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* Deceased

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CURRENT TRENDS IN RESEARCH AND CLINICAL MANAGEMENT OF DIABETES*

Conference Chairman and Consulting Editor

PETER H FORSHAM

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Part III. Biochemistry and Physiology of Insulin and the Sulfonylurea Compounds

Part IV. The Status of Oral Agents in the Therapy of Diabetes

INTRODUCTION

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The collaboration of an active group of investigators to produce this mono- [illegible]

An acceptable definition of the various types of hyperglycemia has become of increasing practical importance with the inclusion of the sulfonylureas in the therapeutic armamentarium against diabetes Naunyn already described three different types of diabetes at the turn of the century in his classic text entitled *Der Diabetes Melitus*[1] This investigator differentiated between (1) the diabetes of young patients, mostly before the thirtieth to fortieth year, "the pure diabetes"; (2) adult diabetes, mostly "a mild disease of older patients"; and (3) the organic diabetes associated with conditions such as cirrhosis. This classification has taken on practical meaning as the prematurity-onset diabetic will not respond to sulfonylureas, whereas the postmaturity-onset diabetic will usually do so

The genetics of diabetes is becoming increasingly more important, since we have interfered with natural selection with the advent of insulin and are thereby increasing the reservoir of potential diabetics enormously We are now capable of preventing the fatal outcome of coma with a high degree of assurance We can provide patients with at least potentially adequate amounts of insulin on a fairly normal diet, as adjudged by near-normal blood sugar levels much of the time, this may also be done with sulfonylureas and biguanides However, it has become well recognized that, even with the very best "control," changes in the integrity of the vascular system occur in varying degrees in long-continued diabetes Among such changes are, notably, the degenerative ones in the small vessels of the eyes and kidneys and the accelerated atherosclerosis of larger vessels In this monograph contributors discuss progress of the research into this all-important and discouraging phenomenon, which can be solved only by a study of the [illegible] employment These sociological considerations have become the physician's problem as well as the patient's and are also discussed

[illegible]

aspects we finally launch a survey of the present status of the clinical use of hypoglycemic agents other than insulin in the management of the diabetic.

I can find no more suitable introduction to this publication than the statement made by Claude Bernard in 1877.[2] "If in the field of diabetes all the

Part I. Diabetes Mellitus: Certain Fundamental Clinical and Socioeconomic Considerations

THE GENETICS OF DIABETES A REVIEW*

Arthur G Steinberg

Departments of Biology and Preventive Medicine, Western Reserve University, Cleveland, Ohio

According to Foster (1912), the earliest report of a familial incidence of diabetes mellitus was by Morton in 1696. Morton reported a family in which four children were diabetic. It seems reasonable to assume that the familial nature of diabetes was known long before this, because diabetes mellitus was apparently recognized by the sweet taste of the urine as early as 500 A D (Saundby, 1908) Be that as it may, daily experience early convinced clinicians that diabetes mellitus is frequently familial. It was not until the fourth decade of this century, however, that it was quantitatively established that the increased incidence is statistically significant (Pincus and White, 1933) These observations strongly indicated that susceptibility to diabetes is genetically determined because the necessary and sufficient criterion for establishing the presence of a genetic component in the causation of a character (disease) is an increased familial incidence in the absence of environmental factors that can explain this increase

Further evidence for the hereditary nature of diabetes mellitus is offered by [illegible]

complete, hence, postnatal environmental factors are important in determining the occurrence of frank diabetes

Pincus and White (1933) showed that the familial incidence of diabetes may be explained by assuming that susceptibility to diabetes is due to homozygosity for a recessive gene Independently, and at essentially the same time, William Allan (1933) arrived at the same conclusion from his highly skilled and too-frequently overlooked analysis of the family data from 143 cases

These pioneering studies have been followed by numerous studies of large samples The several investigators have advanced a variety of interpretations [illegible]

Three broad categories of diabetes appear to be recognized (1) juvenile, with acute onset, severe course, and sensitivity to insulin, (2) late, with gradual onset, generally in obese individuals, mild course, and often no need of insulin;

* The work reported in this paper was supported in part by Research Grants from the National Heart Institute and the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md

pathological obscurities have not as yet been completely elucidated, this is because the knowledge of the normal function is still imperfectly understood. Only by increasing our knowledge of the physiological aspects shall we find the basis for the formulation of an adequate pathological theory and by this dual approach we shall eventually master the control of the morbid phenomena. Physiology defined in this framework will be the only certain guide to the understanding of the pathology and the therapy of diabetes mellitus" It is in the tradition of this great man that the contributors to this monograph, representing all levels of investigation, basic and applied, furnish an impetus to the advancing knowledge of diabetes and its complications.

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1 Naunyn, B 1906 Der Diabetes Mellitus 2nd ed A. Holder, ed. Vienna, Austria
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Further evidence for the hereditary nature of diabetes mellitus is offered by the many studies of twins (Then Berg, 1938, Joslin and White in Joslin *et al*, [illegible]

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These pioneering studies have been followed by numerous studies of large samples The several investigators have advanced a variety of interpretations to explain their data Prominent among them are hypotheses that distinguish the early-onset, severe, brittle diabetes from the late-onset, mild, stable type These hypotheses are attractive on a priori grounds because of the clinical heterogeneity of the disease

Three broad categories of diabetes appear to be recognized (1) juvenile, with acute onset, severe course, and sensitivity to insulin, (2) late, with gradual onset, generally in obese individuals, mild course, and often no need of insulin,

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and (3) J-type, with onset in young, thin individuals, insensitivity to insulin, and generally absence of ketotic reactions (Hugh-Jones, 1955)

The heterogeneity of the disease leads geneticists to expect that more than one genetic mechanism is concerned in the determination of susceptibility to diabetes. This suspicion is based on the experience that physiologically different characters are determined by different genes and, vice versa, that different genes causing similar appearances (phenotypes in genetic terms; syndromes, diseases, abnormalities in clinical terms) do so by different biochemical or physiological mechanisms.

Insofar as I am aware, no genetic studies have been made of the J type of diabetes, but many have been made of the other two varieties.

I have already mentioned the work of Pincus and White (1933, 1934) and of Allan (1933). Cammidge (1929, 1934) examined the pedigrees of 1000 diabetic patients He concluded that early-onset diabetes is due to a recessive gene, and that late-onset diabetes is due to a dominant gene, presumably these are not alleles Hanhart (1950) believes that all diabetic pedigrees may be explained by assuming recessive inheritance due to genes at one or more genetic loci, but neither Hanhart nor Cammidge has employed quantitative methods to analyze his material Penrose and Watson (1945) reported the presence of a sex-linked tendency in susceptibility to diabetes, but Thompson and Watson (1952), in a subsequent study on a considerably larger sample that included at least a part of the sample studied by Penrose and Watson, failed to confirm the finding of a sex-linked tendency [illegible]
tors have also failed to confirm [illegible]
concluded that their data were " [illegible]
somal recessive gene "

Ilse von Kries (1953), in a study of the families of 1305 probands, concluded, in agreement with Levit and Pessikova (1934), who studied the families of [illegible]

[illegible]

diabetes among the parents of the probands was about equal to that among [illegible]

[illegible]

Grunnet (1957) published an extensive monograph based on his study of the [illegible]

[illegible]

cases, but [illegible] diabetes mellitus must be presumed

"A part of the mild cases of diabetes in elderly individuals are probably exogenically conditioned

"The majority of all cases of diabetes mellitus, in any case the majority of

the severe cases, must be regarded as one group of primary pancreatogenic diabetes, probably inherited recessively."

The reasons for Grunnet's conclusions remain obscure to me At no time are the data subjected to genetic analysis, not even to the simple extent of comparing the frequency of diabetes among the sibs of those with an affected parent with that among the sibs of those with healthy parents. Unfortunately, despite about 65 pages of tables and graphs, it is not possible to determine the number of sibs of each patient or the distribution of family size The data therefore must remain unanalyzed

Genetic Pattern

I have elsewhere analyzed in detail the studies published prior to 1952 (Steinberg and Wilder, 1952*b*) The objection to the conclusion that diabetes mellitus is due to a dominant gene rests upon the observation that, in all sets of data that are presented so that the comparison can be made, it is found that twice as many of the probands' sibs are diabetic if one parent is diabetic than if neither parent is diabetic Dominant inheritance does not lead to such a pattern

It is possible to elaborate a two-gene system that would approximate the data. Such a system would require a modifying gene that increases the penetrance of the dominant gene that leads to diabetes Several unknowns that may be manipulated at will are inherent in such a hypothesis Three of these are (1) the frequencies of the alleles at each locus, (2) the penetrance of the [illegible]

planations, especially when we have no way of testing them

Despite these objections to dominant inheritance of diabetes as a generalization, the possibility remains that in rare instances diabetes is due to a dominant gene Burnstein and Patterson (1949) published a remarkable pedigree extending over 5 generations and purporting to show dominant inheritance of a mild diabetes with clear expression of anticipation N Burnstein, Veterans Administration Hospital, Jackson, Miss, generously made it possible for me to correspond with the proband The patient, a registered nurse, recompiled her family history for me by corresponding with various of her relatives I [illegible]

and (3) J-type, with onset in young, thin individuals, insensitivity to insulin,

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twice as frequent among those with an affected parent as among those with nondiabetic parents

It appears from the foregoing that genetic susceptibility to diabetes is unlikely to involve sex-linked genes, or to be due to a dominant gene with incomplete penetrance, or to a gene that leads to late-onset, mild diabetes when heterozygous and to early-onset, severe diabetes when homozygous The only [illegible] ners affected among the parents of individuals homozygous for a recessive gene is as $p^2:2pq:q^2$, respectively, where q equals the frequency of the recessive gene (conveniently, the proportion of affected parents in the sample of parents equals q) These ratios are independent of age at onset, provided the distribution of age at onset is the same for the offspring of the three types of matings, and provided that only a relatively small proportion of the nondiabetic parents may be genetically liable to diabetes These conditions appear to be fulfilled by those samples published in sufficient detail to permit such an analysis

TABLE 2

FREQUENCY (IN PER CENT) OF DIABETES AMONG THE SIBS OF PATIENTS WITH EARLY AND LATE ONSET OF DIABETES

Patient's age at onset	All sibs		Neither parent diabetic		One parent diabetic	
			Sibs		Sibs	
	Total	Per cent diabetic	Total	Per cent diabetic	Total	Per cent diabetic
Harris (1950)						
0–29	1,019	4 1	971	3 5	48	18 8
30–	2,773	4 4	2,446	4 1	327	10 7
Thompson and Watson (1952)						
0–29	482	7 5	425	6 4	57	15 8
30–	4,125	9 1	3,411	7 8	714	15 3
Steinberg and Wilder (1952b)						
0–29	828	6 0	736	5 0	92	14 1
30–	7,456	6 0	5,928	4 6	1,528	11 2

TABLE 3 presents the comparison of the observed and expected numbers of the 3 types of mating for each of 6 sets of data. The χ^2 test of goodness of fit indicates that differences between the observed and expected numbers have a high probability of occurring by chance in 5 of the 6 samples The sixth sample has been shown (Steinberg and Wilder, 1952a) to have certain inconsistencies with reference to ages at onset, which suggest that this set of data may not be as suitable for analysis as the remaining 5 sets. We may conclude, therefore, that the only simple genetic hypothesis consistent with the data is

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the 12
in 5, ar
have become diabetic in an even year of life, and 4 of the 8 with onset in an odd year of life became diabetic in a year of life ending in 5.

The discrepancy in the number of diabetics in the 2 pedigrees has already been mentioned, we are concerned now with the peculiar distribution of the ages at onset In an unbiased sample, equal numbers with onset in odd- and even-numbered years of life would be expected, and only one fifth of those with onset in an odd-numbered year of life would have onset in a year ending in 5 It is apparent that neither of the compilations of this pedigree is free of bias

Mild diabetes may be d...

TABLE 1
FREQUENCY OF DIABETES AMONG THE PARENTS OF PATIENTS WITH EARLY AND LATE ONSET OF DIABETES*

Patient's age at onset (years)	Per cent of diabetic parents	
	Harris	Steinberg and Wilder
0–29	3 3	5 0
30–	6 2	11 4

* Data from Steinberg and Wilder, 1952b

added that the distribution of the homozygous and heterozygous types would ...

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diabete
onset than among the parents ... late onset. Steinberg and Wilder (1952b) showed that this was not the case in either Harris's data or ...

not fulfilled ... is essentially the same, regardless of the age of the proband at onset, and is about

twice as frequent among those with an affected parent as among those with nondiabetic parents

It appears from the foregoing that genetic susceptibility to diabetes is unlikely to involve sex-linked genes, or to be due to a dominant gene with incomplete penetrance, or to a gene that leads to late-onset, mild diabetes when heterozygous and to early-onset, severe diabetes when homozygous The only simple genetic pattern left to consider is recessive inheritance

It can be shown (Dahlberg and Hultkranz, 1927, Steinberg and Wilder, 1952*b*) that the relative frequencies of matings with none, one, and both partners affected among the parents of individuals homozygous for a recessive gene is as $p^2:2pq.q^2$, respectively, where q equals the frequency of the recessive

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FREQUENCY (IN PER CENT) OF DIABETES AMONG THE SIBS OF PATIENTS WITH EARLY AND LATE ONSET OF DIABETES

Patient's age at onset	All sibs		Neither parent diabetic — Sibs		One parent diabetic — Sibs	
	Total	Per cent diabetic	Total	Per cent diabetic	Total	Per cent diabetic
Harris (1950)						
0–29	1,019	4.1	971	3.5	48	18.8
30–	2,773	4.4	2,446	4.1	327	10.7
Thompson and Watson (1952)						
0–29	482	7.5	425	6.4	57	15.8
30–	4,125	9.1	3,411	7.8	714	15.3
Steinberg and Wilder (1952b)						
0–29	828	6.0	736	5.0	92	14.1
30–	7,456	6.0	5,928	4.6	1,528	11.2

gene (conveniently, the proportion of affected parents in the sample of parents equals q) These ratios are independent of age at onset, provided the distribution of age at onset is the same for the offspring of the three types of matings, and provided that only a relatively small proportion of the nondiabetic parents may be genetically liable to diabetes. These conditions appear to be fulfilled by those samples published in sufficient detail to permit such an analysis

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that susceptibility to diabetes is due to the homozygous condition of a recessive gene

The way the data have been collected does not permit us to decide whether only one gene is involved or whether homozygosis for any one of two or more recessive genes may lead to diabetes. While it is a simple matter in experimental genetics to distinguish among these alternatives, it is difficult to do so in human genetics, particularly for a character with variable age at onset.

The method most likely to distinguish between the alternatives of a single locus versus two or more loci is to follow the offspring of families in which both

TABLE 3

COMPARISON FOR 6 SETS OF DATA OF EXPECTED NUMBERS OF EACH OF 3 KINDS OF MATINGS YIELDING DIABETIC OFFSPRING WITH THE OBSERVED NUMBERS. THE EXPECTED NUMBERS ARE COMPUTED ON THE ASSUMPTION OF RECESSIVE HEREDITY

Mating	Steinberg and Wilder (1952b)		Pincus and White (1933)		Allan (1933)		Harris (1950)		Thompson and Watson (1952)		von Kries (1953)	
	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
Neither parent diabetic	1589	1588.6	440	440.6	124	122.8	1124	1119.1	1404	1408.2	1137	1135.0
One parent diabetic	370	370.8	80	78.8	17	19.4	109	118.8	223	214.6	160	164.1
Both parents diabetic	22	21.6	3	3.6	2	0.8	8	3.1	4	8.2	8	5.9
Total	1981	1981.0	523	523.0	143	143.0	1241	1241.0	1631	1631.0	1305	1305.0
$\chi^2_{(1)}$	0.009		0.119		2.109		8.573		2.492		0.885	
P	>0.90		>0.70		>0.10		<0.01		>0.10		>0.30	

V. A. Newill, G. F. Badger, A. S. Littell, M. B. Sussman, and I are undertaking such a study as part of a broader study of diabetes. Others have attempted such studies in the past (Pincus and White, 1934; Steiner, 1936; Hanhart, 1950), but the data are not presented in sufficient detail to permit their evaluation.

am aware, ... the glucose tolerance test to test a genetic hypothesis. These investigators tested the nondiabetic sibs of patients with none, one, or both parents diabetic ... frequencies of abnormal glucose tolerance tests among these sibs were

recessive inheritance The observed frequencies, however, fall short of the expected frequencies, which suggests that the test does not detect all presumed prediabetics. It would be of interest to know how the frequency of abnormal glucose tolerance tests varies with the age of those tested Unfortunately, Pincus and White did not publish such data A similar omission occurs in the data published by those using the cortisone-stressed glucose tolerance test (Conn, 1958; West, 1957)

If we accept the hypothesis that most if not all cases of diabetes are due to homozygosis for the same recessive gene, we can estimate the frequency of the gene in the population (Steinberg and Wilder, 1952*b*) and, using this estimate, the probability that various relatives of a diabetic patient would be liable to diabetes (Steinberg, 1955*a*, *b*). It is estimated that the gene frequency is about 22 per cent, and that about 5 per cent of our population is genetically

TABLE 4

PROBABILITY THAT AN INDIVIDUAL WILL BE GENETICALLY LIABLE TO DIABETES IF HE HAS 1 OR MORE DIABETIC RELATIVES*

Probability that a person is genetically liable to diabetes†	Diabetic relatives
Up to and including 20 per cent	(1) First cousin, (2) uncle or aunt, (3) one grandparent, (4) two [illegible]
30 to 40 per cent	[illegible]
50 to 80 per cent	[illegible]

* [illegible]

liable to diabetes Since only about 2 per cent of the population is diabetic (detected and undetected, Joslin *et al*, 1946), it follows that the frequency of the disease may be expected to increase as our population ages TABLE 4, from Steinberg (1955), presents a summary of the probability of being genetically liable to diabetes (*dd*) as a function of the relationship to a diabetic patient The risk runs from less than 20 per cent to 80 per cent The table does not include the risks incurred when a sib is diabetic because these are clearly 25, 50, and 100 per cent if neither, 1, or both parents, respectively, are diabetic

While it is of some use to be able to offer probabilities of being liable to diabetes, [illegible]

[illegible] years per generation, and that a child would rarely if ever become diabetic at a greater age than had his parent. This is known as anticipation Steinberg and Wilder (1950 and 1952*a*) showed that, among

children of diabetics, the younger age at onset of diabetes is a statistical and not a biological phenomenon. These conclusions were based on an analysis of 200 parent-child pairs for which the ages at onset were known for both parent and child

In their paper on the genetics of diabetes, Steinberg and Wilder (1952*b*) presented data for 301 parent-child pairs, these include the 200 pairs previously

TABLE 5

NUMBER OF PATIENTS WHOSE AGE AT ONSET WAS IN A DECADE PRIOR TO THAT AT ONSET OF THE DIABETIC PARENT*

Age of parent at onset	Total No of parent-child pairs	No of patients with onset in a prior decade of life
20 to 29	3	1
30 to 39	15	5
40 to 49	51	18
50 to 59	93	43
60 to 69	85	71
70 to 79	41	40
80 to 89	13	13
Total	301	191

Per cent prior onset = (191/301) × 100 = 63 4

* Data from Steinberg and Wilder 1952*b*.

TABLE 6

DERIVATION OF THE EXPECTED FREQUENCY OF PRIOR ONSET AMONG 301 PATIENTS (VALUES ARE PERCENTAGES)

Age in years	Cumulative distribution of age at onset*	Distribution of age at onset among 301 parents	Expected frequency of prior onset in the children
0 to 9	4 9	—	
10 to 19	11 8	—	
20 to 29	19 4	1 0	0 1
30 to 39	31.6	5 0	1.0
40 to 49	54 1	16 9	5 3
50 to 59	81 1	30 9	16 7
60 to 69	96 4	28 2	22 9
70 to 79	98 8	13 6	13 1
80 to 89	100 0	4 3	4 2
Total		99 9	63 3

* Based on data of 12,740 patients published by Joslin *et al* (1946).

reported (1952*a*). TABLE 5 presents a summary of these 301 pairs Onset was in a prior decade of age in 63 4 per cent of the offspring. Hence, as Woodyatt and Spetz reported, prior onset occurs in the majority of the diabetic

children with prior onset for each decade of onset in the parent is obtained by multiplying the value for the proportion of parents with onset in a given decade by the percentage of all diabetics with onset before that decade Thus, for parents with onset during the fifth decade (40 to 49 years), the expected percentage of children with prior onset is $0\ 169 \times 31\ 6 = 5\ 3$ per cent If this operation is repeated for sets of parents with onset in each of the decades, the total expected frequency may be derived This frequency, 63 3, is almost identical with the observed frequency, 63 4

TABLE 7
AGE AT ONSET AND FREQUENCY OF PRIOR ONSET VERSUS DIABETIC PARENT

Diabetic patients	Diabetic parent	
	Mother (186)	Father (115)
Percentage with prior onset	63 4	63 5
Mean age at onset in years (a)*	49	48
Mean age at onset in years (b)†	42	39

* (a) All patients with a diabetic parent
† (b) Patients whose diabetic parents became ill before age 50 44 mothers, 23 fathers

TABLE 8
FREQUENCY OF DIABETES AMONG THE SIBS OF DIABETIC PATIENTS AS A FUNCTION OF THE SEX OF THE DIABETIC PARENT*

Diabetic parent	Number of patients	Sibs		
		Total	Diabetic	
			No	Per cent
Mother	220	1000	113	11 3
Father	150	620	72	11 6
Total	370	1620	185	11 4

* Data from Steinberg and Wilder (1952*b*)

It has been shown that neither the diabetic nor the prediabetic state in the mother influences the age at onset or the probability of occurrence of diabetes in the child (Steinberg and Wilder, 1952*a*, Steinberg, 1955*b*) TABLE 7 presents

betic parent.

In this sample the mean difference between the ages at onset of the parents and the probands is 19 years It may be shown by calculations similar to, but more detailed than, those illustrated in TABLE 6 that the expected advance in age (assuming independence of age at onset of parent and child) is also 19 years (Steinberg and Wilder, 1952*a*)

children of diabetics, the younger age at onset of diabetes is a statistical and not a biological phenomenon These conclusions were based on an analysis of 200 parent-child pairs for which the ages at onset were known for both parent and child

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0 to 9	4 9	—	
10 to 19	11.8	—	
20 to 29	19 4	1 0	0.1
30 to 39	31.6	5 0	1 0
40 to 49	54 1	16 9	5 3
50 to 59	81.1	30 9	16 7
60 to 69	96 4	28 2	22 9
70 to 79	98.8	13 6	13 1
80 to 89	100 0	4 3	4 2
Total		99 9	63 3

* Based on data of 12,740 patients published by Joslin *et al* (1946)

reported (1952*a*). TABLE 5 presents a summary of these 301 pairs. Onset was in a prior decade of age in 63 4 per cent of the offspring Hence, as Woodvatt and Spetz reported, prior onset occurs in the majority of the diabetic

parent and child

The calculations are illustrated in TABLE 6 The expected frequency of

PINCUS, G & P. WHITE 1934 On the inheritance of diabetes mellitus III Am J Med Sci. 188 782–790

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We may conclude, therefore, that anticipation has no biological significance, and that we cannot use the parent's age at onset to predict the child's age at onset or the end of the period of risk for the child

If we assume that the distribution of the age at onset is essentially the same for all segments of the population of the United States, we may use the cumulative distribution of the age at onset shown in column 1 of TABLE 5 to estimate the proportion of the period of risk that has been passed. For example, 54 per cent of those who will become diabetic are already diabetic by the age of 50 years, therefore, we may assume that 54 per cent of the risk has been run by this age For the present, this appears to be about as well as the geneticist can do More precise information for the patient will become available only when the prediabetic can be detected with a high degree of certainty.

Summary

It is demonstrated that susceptibility to diabetes is probably inherited as a

phenomenon

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diabetes on the occasional curve that drops abruptly to normal at 1½ hours

within the shaded zone of FIGURE 1. It should be emphasized that these criteria for the interpretation of the glucose tolerance test are applicable only in ambulatory and otherwise healthy individuals

The criteria described are similar to those of Moyer and Womack[3] and those

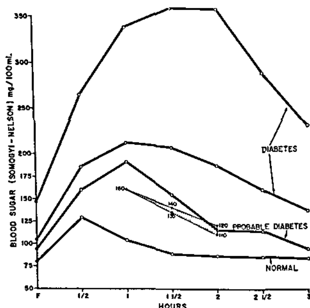


FIGURE 1 Criteria used for the interpretation of the standard oral glucose tolerance test

of Mosenthal and Barry[4] However, our criteria are less restrictive than those of Mosenthal and Barry, who considered carbohydrate tolerance to be abnormal when the maximum blood sugar value is above 150 mg /100 ml and the 2-hour value above 100 mg /100 ml Also, these workers did not employ a value at 1½ hours in mildly abnormal glucose tolerance tests to eliminate false positive rebound curves

It is obvious that any criteria which employ a sharp dividing line between normal and abnormal must be arbitrary What, then, are the justifications that make us believe that our criteria are sound? Applying these criteria in

THE EARLY RECOGNITION OF DIABETES MELLITUS*

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Progressively earlier recognition of the diabetic state is vital if progress is to be made toward the eventual control and prevention of the disease. The presence of mild diabetes mellitus may remain unrecognized in a large number of individuals for many years unless diagnostic laboratory procedures are freely employed in groups of individuals in whom experience has shown a high incidence of latent diabetes Dependable diagnostic criteria are now available that make it possible to detect these previously unsuspected diabetic individuals In addition, it is hoped that other means for detection of the diabetic state will be discovered that will give earlier evidence of its existence than is possible by present methods of testing

This discussion has two purposes, first, to consider the use and the criteria for the interpretation of the standard oral glucose tolerance test employed for the early detection of diabetes and second, to consider experience with the cortisone-glucose tolerance test as used for the possible prediction of future diabetes mellitus

The early recognition of diabetes mellitus depends upon the use and interpretation of proper laboratory procedures. In some cases the presence of

We have used the oral glucose tolerance test in our studies For at least 3 days preceding the test the subjects ingest a diet containing approximately 300 gm carbohydrate per day plus maintenance calories[1] Although the in-

The criteria that we employ for

cose tolerance test are illustrated

* The work reported in this paper was supported in part by Research Grant A 888 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md

Several types of glucose tolerance curves that cannot be classified as indicating diabetes or probably diabetes according to our criteria, but which nevertheless may have prognostic significance, deserve comment The diagnosis of diabetes mellitus cannot be made with confidence on the basis of an abnormal elevation of the blood sugar level at ½ or 1 hour if it is accompanied by a normal 2-hour blood sugar level during the glucose tolerance test Rapid transit of

TABLE 3
PATIENT A C, MALE, HEIGHT 6'0", GLYCOSURIA FOUND TWICE IN 1930

Year	Age	Weight	Glucose tolerance test (hours)			
			Fasting	1	2	3
1934	48	170	0 86	+ 205	+++ 115	++ 49
1953	67	182	Polyuria, polydipsea, 7-pound weight loss during preceding month			
11-17-53			++++ 220			
12-10-53			+++ 232			

TABLE 4
PROGNOSTIC IMPORTANCE OF ABNORMALLY HIGH PEAK BLOOD SUGAR LEVELS WITH NORMAL 2-HOUR LEVELS

Subject and height	Date	Age	Weight	Glucose tolerance test (hours)						
				Fasting	½	1	1½	2	2½	3
M P, male, 6'	1935	43	160	0 130		++++ 174		++++ 93		0 68
	1940	48	161	++++ 211						
A M, female, 5'5"	3-5-53	33	160	0 100		++ 180		+ 96		+ 118
	6-25-53	33	162	123	214	259	189	158	163	137
	3-23-59	39	148	136	240	280	264	180	193	137
J G, male, 5'10"	3-15-55	24	178	0 98	+++ 198	+++ 153		+++ 76		0 56
	6-1-55	24	177	0 88	188	+++ 207	+++ 214	+++ 218	143	+++ 140

sugar levels during the glucose tolerance test is indicated TABLE 4 shows the [illegible]

dividuals to be no more frequent than in the general population However,

abnormalities of glucose tolerance frequently shows further impairment of carbohydrate utilization clearly diagnostic of diabetes mellitus. Decompensation of carbohydrate tolerance may occur rapidly, although in middle-aged individuals loss of glucose tolerance may be only slowly progressive over many years The following examples will serve to illustrate the significance of mild abnormalities of glucose tolerance as defined by our criteria

Subject A N (TABLE 1) shows a 1-hour blood sugar value of 177 mg /100 ml and a 2-hour value of 121 mg /100 ml A few days later, fasting hyperglycemia was diagnostic of diabetes mellitus Patient K. C. (TABLE 2) is an example of "probable diabetes," since the 2-hour value is between 100 and 120/ mg /100 ml Nine years later no doubt remains about the diagnosis of dia-

TABLE 1
PATIENT A N, MALE, AGED 69 YEARS, HEIGHT 5'8", WEIGHT 160 LB

Date	Glucose tolerance test (hours)					
	Fasting	½	1	1½	2	3
9-14-58	94	171	177	188	121	143
9-17-58 (surgery)	124					
9-18-58	171					

TABLE 2
PATIENT K C, MALE, HEIGHT 6'1", FAMILY HISTORY OF DIABETES (MOTHER)

Year	Age	Weight	Glucose tolerance test (hours)									
			Fasting	½	1	1½	2	2½	3	3½	4	4½
1948	36	160	76		197		118	96	54	36	58	68
1957	45	183	119 115 100	208	286	260	216	(Diet discontinued by patient after 3 months)				
1958	46	191	96	177	216		288		179			

betes mellitus. In subject A C (TABLE 3) the diagnosis of probable diabetes

mellitus.[5]

using the same criteria in a control group of 127 subjects without a family history of diabetes or of large babies, only one previously unrecognized diabetic (Curve 2) and one probable diabetic (Curve 3) were discovered. The other 125 subjects, (more than 98 per cent) had normal glucose tolerance tests. Thus, at least a 19 per cent incidence of previously unsuspected diabetes can be found

TABLE 6
REVERSIBILITY OF DIAGNOSTIC GLUCOSE TOLERANCE TEST
B. S., male height 5'6", weight 152 lb
3-16-38 Glucosuria found, but no symptoms

Date	Age	Glucose tolerance test (hours)			
		Fasting	1	2	3
3-18-38	24	0 214	375	353	273
3-29-38	24	0 107	146	112	99
1946	32	Furunculosis, polydipsia, polyphagia, 8 lb weight loss, recovered without treatment			
1947	33	Abdominal wall abcess, fatigue *FBS*—350 mg / 100 ml. Diagnosis: Diabetes mellitus. Diet and insulin			
1948	34	Retinitis proliferans, vitreous hemorrhages			

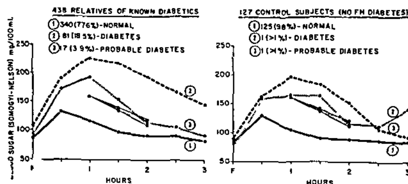


FIGURE 2. Standard oral glucose tolerance tests

in close relatives of diabetic patients, while this incidence is less than 1 per cent in the control group. A very much higher incidence of previously unrecognized diabetes will be found if studies are conducted among close relatives of diabetic patients than in the general population.

To develop means of recognizing the potential diabetic is the aim of our current investigations. The cortisone-glucose tolerance test has been used as a test for determining the potentiality for the development of the disease

we have found unsuspected diabetes in 63 per cent of the patients with nondiabetic glycosuria in whom follow-up glucose tolerance tests were performed up to 30 years later.[4] Approximately one third of the group had initially exhibited fasting glycosuria. This fact

Mild variations of glucose tolerance may be encountered when the test is repeated at intervals of more than a few days in the same individual. In general, however, there is a high degree of reproducibility when the oral glucose tolerance test is carefully performed in conjunction with the use of a standard high-carbohydrate preparatory diet and a true blood sugar method. Occasionally one encounters a rapid change from an abnormal glucose tolerance test to a normal one. It has been recognized for many years that reduction of body weight in the obese middle-aged diabetic patient may result in a

TABLE 5

TRANSITION FROM NONDIABETIC GLYCOSURIA TO DIABETES MELLITUS

Subject and height	Year	Age	Weight	Glucose tolerance test (hours) Fasting	½	1	1½	2	2½	3
A B, female, 5'4"	1936	22	114	0 89		+++ 135		++ 104		0 64
	1948	34	115	++++ 260	(Polyuria, polydipsea, weight loss)					
F J, male, 6'0"	1937	48	190	++ 97		++++ 107		++ 91		++ 68
	1956	67	190	++ 97	+++ 188	+++ 186	++++ 151	+++ 136	++++ 127	+++ 102

return to normal of the glucose tolerance test.[7] It is less well recognized that ... ce test to normal may also ... from a glucose tolerance ... o a normal one does not invalidate these criteria per se. An extreme example of such a change to a

... the 438, 81 (19 per cent) were found to be diabetic. Curve 2 is the composite curve for these previously unsuspected diabetics. Seventeen of the 438 relatives (4 per cent) gave curves indicating probable diabetes (curve 3). In contrast,

When this test is applied to nondiabetic relatives of diabetic patients it may serve as a means for predicting future diabetes.[2, 8, 9]

We used the following technique for the performance of the cortisone-glucose tolerance test in our studies. An individual weighing less than 160 lb receives orally 50 mg. of cortisone acetate 8½ hours and again 2 hours before the ingestion of glucose. If body weight exceeds 160 lb., 62½ mg. cortisone acetate is given orally at the same time intervals. A value of 140 mg. per cent at 2 hours is the critical level for interpretation of the cortisone-glucose tolerance test. Thus, a cortisone-glucose tolerance test giving a 2-hour blood sugar level of 140 mg. per cent or above (and therefore lying at or above the line connecting the points of 160 mg. per cent at 1 hour and 140 mg. per cent at 2 hours in FIGURE 3), represents a "positive response," while a curve with a level of below 140 mg. per cent at 2 hours is regarded as a "negative response."

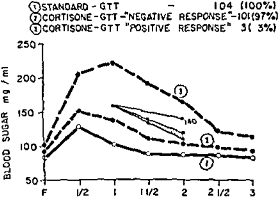


FIGURE 3. Cortisone glucose tolerance tests on 104 healthy subjects with no family history of diabetes or large babies.

FIGURE 3 illustrates our findings with the cortisone-glucose tolerance test in a group of 104 control subjects without a family history of diabetes or large babies. Curve 1 demonstrates their composite standard glucose tolerance test before cortisone was given. After administration of cortisone 101 subjects or 97 per cent of the control group gave "negative responses" to this test with a mean 2-hour blood sugar of 104 mg./100 ml. (Curve 2). Three subjects (3 per cent) of the group gave a "positive response" to this test.

Cortisone-glucose tolerance tests were performed in 295 nondiabetic relatives of known diabetics. All of these subjects had normal control glucose tolerance tests as shown in FIGURE 4. After administration of cortisone 220 of the 295 individuals (75 per cent) showed a negative response with a mean 2-hour blood sugar level of 106 mg. per cent, a response seen in 97 per cent of the control group. Seventy-five of the 295 subjects (25 per cent) showed a positive response. Thus, 25 per cent of the group of nondiabetic relatives respond to this test as do only 3 per cent of the control group.

FIGURE 5 shows the data obtained with 17 subjects classified by their initial

glucose tolerance test as individuals with probable diabetes (Curve 1). Fifteen of the 17 (88 per cent) gave positive responses to the cortisone-glucose tolerance test, while 2 of the 17 gave negative responses to it.

FIGURE 6 demonstrates data obtained in 22 obese, mild diabetic patients Curve 1 shows the composite glucose tolerance test before reduction of body weight After weight reduction all subjects exhibited normal standard glucose tolerance curves, as shown in Curve 2. Cortisone-glucose tolerance tests

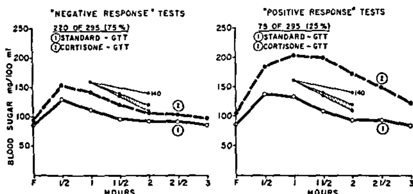


FIGURE 4 Cortisone glucose tolerance tests on 295 nondiabetic relatives of diabetic patients

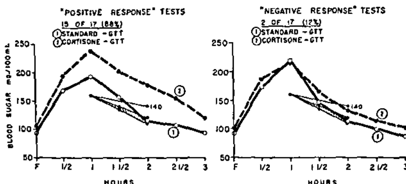


FIGURE 5 Cortisone-glucose tolerance tests on 17 subjects with probable diabetes

were then performed Of the 22, 19 (86 per cent) gave a positive response to the test (Curve 3)

In correlating these results it seems very significant that 25 per cent of the group of nondiabetic relatives of diabetic patients respond to this test in the same way as do 88 per cent of patients with probable diabetes and 86 per cent of the group of obese diabetics whose carbohydrate tolerance had returned to normal after reduction of body weight On the other hand, only 3 per cent of the control group without a family history of diabetes gave a positive response to the cortisone-glucose tolerance test.

cose tolerance test in our studies. An individual weighing less than 160 lb receives orally 50 mg of cortisone acetate 8½ hours and again 2 hours before the ingestion of glucose. If body weight exceeds 160 lb., 62½ mg. cortisone acetate is given orally at the same time intervals. A value of 140 mg. per cent at 2 hours is the critical level for interpretation of the cortisone-glucose tolerance test Thus, a cortisone-glucose tolerance test giving a 2-hour blood

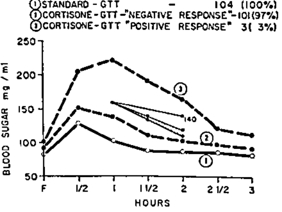


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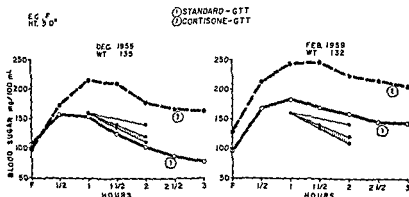


FIGURE 8 Glucose tolerance tests and cortisone glucose tolerance tests in a subject with familial diabetes mellitus (mother)

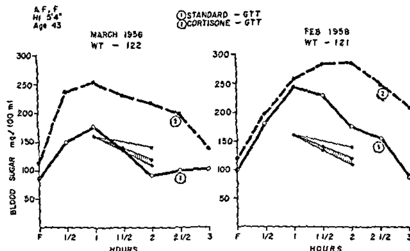


FIGURE 9 Glucose tolerance tests and cortisone glucose tolerance tests in a subject with familial diabetes mellitus (mother, 5 siblings)

each of the two groups Such studies have been in progress in 91 nondiabetic relatives of diabetic patients over the last 6 years (TABLE 7)

Of the 91 subjects, 34 had positive responses to the cortisone-glucose tolerance test at the time of the initial tests Subsequently, 6 (18 per cent) of these 34 subjects have developed diabetes, and another 4 (12 per cent) have probable diabetes Of the 57 subjects with negative responses to the cortisone-

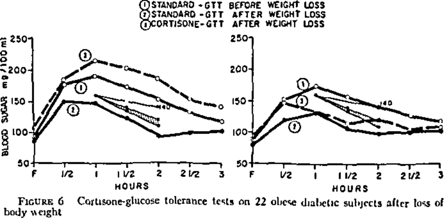


FIGURE 6 Cortisone-glucose tolerance tests on 22 obese diabetic subjects after loss of body weight

TABLE 7
FOLLOW-UP (1 TO 6 YEARS) RESULTS IN 91 NONDIABETIC RELATIVES OF DIABETIC PATIENTS

Initial normal GTT		Progression to		
Cortisone-GTT	Number subjects	Diabetes number	Probable diabetes number	Total abnormal number
Positive response	34	6 (18%)	4 (12%)	10 (30%)
Negative response	57	1 (2%)	0	1 (2%)

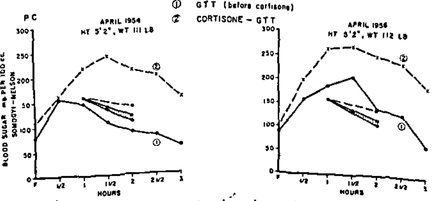


FIGURE 7. Glucose tolerance tests and cortisone glucose tolerance tests in a 32 year old female patient with familial diabetes mellitus (father and brother).

PREGNANCY AND THE PREDIABETIC STATE

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Quite appropriately it has been said that the ideal clinical state for the investigation of "prediabetes" is to be found in the pregnant woman[1] Most of our knowledge and postulation have come from studies of her "career" and her offspring

The investigations of Jackson,[2] Conn,[3] and Hoet[4] refer respectively to in-

[illegible]

A basic premise by which an investigation might be guided may be that those who ultimately become overtly diabetic are born with a susceptibility or predisposition to the disorder, transmitted through heredity and/or by an abnormal metabolic intra-uterine environment With the passage of time a susceptible human host ages, being subjected to cumulative environmental organic stresses and insults, including such things as obesity and infection and, if a woman, possibly subjected to the stress of repeated pregnancies The time at which an irreversible state of diabetes mellitus will manifest itself may depend upon the extent of the basic pattern of susceptibility and the amount of pathology produced by each added stress or physiological insult

Our present interest is concerned with pregnancy and the prediabetic state In this regard several studies[5-8] made in retrospect and reported in medical literature conclude that the diagnosis of overt diabetes in many women is not made until months or even many years after these women have had certain characteristic experiences in pregnancy These experiences, allegedly delineating the "prediabetic state," include a high incidence of big babies, stillbirths, neonatal deaths, spontaneous abortions, premature deliveries, toxemia, and congenital anomalies

As a part of our premise, it is also felt that, during pregnancy, transient abnormalities of carbohydrate metabolism as detected by a standard oral glucose tolerance test may indicate that a diabetogenic effect is being exerted, not only on the mother, but also on the fetus

Some of the abnormal experiences in pregnancies related subsequently to the prediabetic state are more pronounced than others While the expected in-

[illegible]

betes in the mother[9]

Fetal and neonatal mortality is reported in the high range of 20 to 40 per cent in the 5-year period immediately preceding the development of overt diabetes, but it is increased appreciably over normal as much as 30 years prior to the recognition of this metabolic disorder[8]

glucose tolerance test, only 1 has developed diabetes. In summary, 30 per cent of the positive-response group has developed diabetes or probable diabetes, while only 2 per cent in the negative-response group yielded an abnormal glucose tolerance test at a later date. Thus, *the cortisone-glucose tolerance test may prove to be of value as a test for the prediction of future diabetes.* FIGURES 7 and 8 give examples of the progression from a normal glucose tolerance, but abnormal cortisone-glucose tolerance, test to diabetes mellitus in the course of

possible prognostic importance of an elevated peak blood sugar level.

Summary

The diagnostic criteria that we employ for the interpretation of the standard oral glucose tolerance test have been reviewed.

Unsuspected diabetes has been found in 19 per cent of 438 close relatives of diabetic patients, while another 4 per cent fell ...

... have been

Positive responses to the cortisone-glucose tolerance tests have been obtained in 3 per cent of the control group without a family history of diabetes, in 25 per cent of the nondiabetic relatives of diabetic patients, in 88 per cent of patients with probable diabetes, and in 86 per cent of a group of obese diabetic patients whose carbohydrate tolerance had returned to normal after reduction of body weight

A positive response to the cortisone-glucose tolerance test in nondiabetic individuals can be interpreted as indicating a *potentiality* or susceptibility for the development of diabetes Follow-up in 91 nondiabetic relatives of diabetic patients over 1 to 6 years supports this view

References

White[10] reports toxemia in 10 per cent of cases, abortions in 42 per cent and perinatal loss in 30 per cent in the 10-year period immediately prior to as well as following the recognition of diabetes.

An increased incidence of congenital defects has been noted by some authorities, but not by others.[6]

The possibility that experiences such as those mentioned above could be harbingers of the ultimate disease is worthy of investigation. If the "prediabetic state" in pregnancy can be recognized by any one or combination of the phenomena alluded to, then early intervention to prevent or halt its progression can be attempted. One preventive measure considered for study is that the correction of abnormal metabolism during pregnancy by insulin administration may reduce some of the complications of pregnancy and/or delay the onset of diabetes.[4, 8]

Objectives and Plan of Study

Since 1954, in cooperation with the Massachusetts Department of Health, Boston, Mass., the Public Health Service of the Department of Health, Education, and Welfare, Washington, D. C.,[11] has conducted at the Boston City [illegible]

making available improved service facilities for the mothers and children in the project.

Three specific objectives are being sought. The first is to determine, if possible, the facts about glucose tolerance tests for abnormal CHO metabolism or "prediabetes" in pregnancy, that is:

(1) If women having such abnormal tests actually have a higher rate of oversize babies, fetal wastage, and complications of pregnancy than those with normal tolerance.

(2) If such women actually do develop frank diabetes in subsequent years [illegible]

[illegible]

Third, it is hoped that a generally practical method of detecting such a "prediabetic state," if it actually exists, will evolve from the study as it is being performed.

At the time pregnant women register for prenatal care they are given a lemon-flavored drink of 50 gm. glucose. Immediately following this a history is taken of past pregnancy experiences and the occurrence of diabetes in the family. One hour after the glucose drink a venous specimen of blood is taken [illegible]

gave 9.7 per cent false positive results) Of women with a history of a large baby, 13 5 per cent had abnormal glucose tolerance tests, as compared with 8 9 per cent of those women who had other positive pregnancy history and 11.5 per cent of the women who had a history of diabetes in the family.

If we consider all of those who may be suspected by at least 1 criterion, only 10 7 per cent would have an abnormal glucose tolerance test This per-

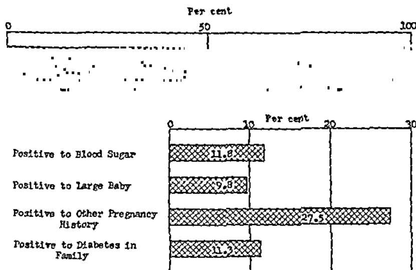


FIGURE 1 Pregnancy and the prediabetic state screening results by various criteria from July 19, 1954 to December 31, 1957 A total of 11,352 pregnancies was screened

TABLE 1
GLUCOSE TOLERANCE TEST RESULTS BY EACH SCREENING CRITERION
(July 19, 1954 to December 31, 1957)

Screening criteria	Glucose tolerance tests			Sensitivity rate	Specificity rate
	Number performed	Positive			
		Number	% of No performed		
Positive to blood sugar	1085	255	23 5	44 9	90 3
Positive to large baby	907	122	13 5	21 5	90.8
Other positive pregnancy history	2482	221	8 9	38 9	73.6
Diabetes in family	1061	122	11 5	21 5	89 1
Positive to at least one criterion	4065	434	10 7	76 4	57 7
Negative to all criteria*	5082	134	2 6	—	—
Total screenees with GTTs	9147	568	6 2	—	—

* The actual number of GTTs performed on ...

and study: (1) women with abnormal glucose tolerance who receive insulin therapy during pregnancy (*insulin-treated*), (2) women with abnormal glucose tolerance for whom insulin is not prescribed (*positive controls*); and (3) women

in charge

The study includes (1) careful observation of mothers throughout their pre-

erance test of the mother at 6 to 8 weeks *post partum*; (4) well-child care for

Results

I present a few of our results, but in so doing I emphasize that this is a preliminary report, the study is still continuing. Any finding or conclusion at this time is a preliminary one and subject to change as more data are accumulated

Some analysis of data currently is available through December, 1957, and FIGURE 1 shows the screening results by various criteria through that date At that time 11,352 delivered pregnancies had been screened, 44 4 per cent were positive screenees. This ratio between those suspected and those not

he time of
ter taking
" Of all
to one or
more babies weighing ... had other abnormal pregnancy history, as has been defined earlier. Of all pregnant women, 11.3 per cent had a history of diabetes in the family It should be remembered that all of these screening suspect groups overlap to some extent, and a woman may have 1 or any combination of the criteria

tests by each screening
'ere we find that of those
arbohydrate metabolism

but a little more than 5 per cent in each of these groups still had some abnormality in their glucose tolerance tests. A little more than 2 per cent of both insulin-treated and positive control cases were revealed as diabetic by their first *post partum* glucose tolerance tests * All or some of these 2 per cent could have been undetected diabetics prior to pregnancy

With regard to our third specific objective of finding a practical procedure for identifying the pregnant woman who has abnormal carbohydrate metab-

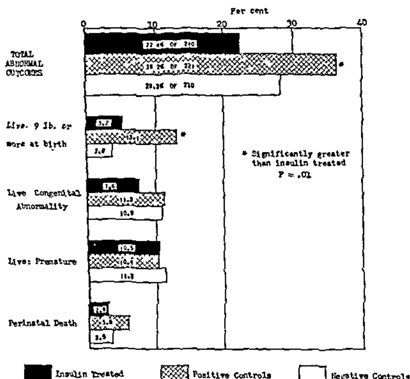


FIGURE 2 Rates of abnormal outcomes for insulin treated patients and positive and negative controls through December 31, 1958

olism or "prediabetes," the most encouraging results so far are those shown in TABLE 3 Here specificity and sensitivity rates for each hour of the glucose tolerance tests are presented Of all the positive screenees who had abnormal glucose tolerance tests when first tested during pregnancy, 99 3 per cent had a 2-hour level of 120 or more mg /100 ml blood This level of sensitivity is satisfactory, but accompanying this good sensitivity is a specificity of only 91 4

* Using the same blood sugar levels shown in our criteria for "abnormal carbohydrate metabolism in pregnancy," a glucose tolerance test outside of pregnancy is considered "diabetic" if any 3 successive levels are reached or exceeded or if both fasting and third hour levels are equaled or exceeded

. . . . much from the percenta
ossibly more select grou
of 790 patients so studie

or about 14 per cent

Of particular interest in TABLE 1 are the specificity and sensitivity ratings screening with at least 1 criterion Screening "positive to at least one criterio gives a sensitivity of 76 4 per cent, by this is meant that our present crite are picking up about 76 per cent of those pregnant women who have an a normal glucose tolerance test during pregnancy However, we are missi about 24 per cent of those whom we would find if we gave such a test to screenees instead of using the present procedure of screening and doing g cose tolerance tests on the positive screenees only Also, we have a 57.7 cent specificity with "at least one criterion positive," meaning that we c rently must restudy about 42 per cent of all women who finally are classif as having negative glucose tolerance tests.

It is estimated that about 6 2 per cent of all screenees would have abnorr carbohydrate metabolism by our criteria if they were all given a glucose t erance test. We performed glucose tolerance tests on a sample of 378 co secutive negative screenees and found that 2.6 per cent had positive resu Applying this percentage to all negative screenees who would have been pected to have a negative glucose tolerance test, we estimate that 134 wo turn out to have a positive glucose tolerance test in spite of screening as norı in every way.

Our research patient case load through December 31, 1958 consists of women who are insulin treated, 231 women who are positive controls, and

FIGURE 2 shows . . . for these 3 research groups Although many of our research cases have gi birth to several children, this bar diagram represents only the analysis of outcome of first pregnancy under our observation Abnormal outcome pregnancy is defined as follows: live baby weighing 9 pounds or more at bi live baby with congenital anomaly, baby alive but premature, or a perin . . . death)

abnormal outcomes occurred am
insulin-treated or negative contr
rence in the outcome of pregnar
There appears to be . . . the insulin treated and the . . .

Conclusions

Let me repeat that I have presented only some of the preliminary findings to date in a very extensive study that is still being continued While there [illegible]

ultimately evolving a practical program of detection of prediabetes in pregnant women that would be suitable for consideration by the medical profession for widespread application It is emphasized, however, that recommendations for such a program must await important refinements needed and a clearer definition of the prediabetic state Also, there must be more evidence of the actual significance, with respect to prevention, that can be attached to the detection of the prediabetic state in pregnancy

Joslin *et al.*[10] have urged us "like Boulin, to stop saying that diabetes is [illegible]

prevention of its complications and its progression to a permanent state I hope that our research project ultimately will shed some clarification clinically and from the standpoint of public health on this confused state of so-called prediabetes in pregnancy.

Acknowledgments

All members of the Chronic Disease Program, Diabetes Field Research and Training, and the Prenatal Metabolism Research Staff have rendered most valuable assistance in the planning, operation, and evaluation of results of this research project

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1 [illegible]

6 [illegible]

7 [illegible]

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9 [illegible]

No. 1

10 JOSLIN, E , H ROOT, P WHITE & A MARBLE *Ibid* : 705

per cent This specificity rating means that 8 6 per cent of the positive screenees who had normal glucose tolerance tests unfortunately, apparently, had some degree of misleading elevation of blood sugar 2 hours after taking 100 gm glucose.

The fact, however, that a single blood sugar analysis following 100 gm of glucose in a standard glucose tolerance test procedure shows such a satisfactory ability to pick up all but 0 7 per cent of the abnormal glucose tolerance cases among those who screened positive encourages us to believe that a simplified and practical method of screening for abnormal carbohydrate metabolism in

TABLE 2
ABNORMAL CARBOHYDRATE METABOLISM IN PREGNANCY
Results of Glucose Tolerance Tests in *Post Partum* Follow-Up of First Study Pregnancy Through December 31, 1958

Result through 5 mos *post partum*	Negative controls		Abnormal CHO metabolism			
			Insulin treated		Positive controls	
	No	Per cent	No	Per cent	No	Per cent
Total completed	173	100 0	180	100 0	191	100 0
Normal	173	100 0	166	92 2	177	92.7
Abnormal	—	—	10	5 6	10	5 2
Diagnosed diabetes	—	—	4	2 2	4	2 1

TABLE 3
SPECIFICITY AND SENSITIVITY RATES OF THE GLUCOSE TOLERANCE TEST BY HOUR OF TEST*
(Using Somogyi Nelson Method)

Hour	Blood sugar level	Specificity	Sensitivity
Fasting	110	99 9	6 1
First	170	99 5	37 8
Second	120	91 4	99 3
Third	110	96 7	82 3

* Based on 3908 GTTs made on women who screened positive through December 31, 1957. A test is considered positive when 2 hourly readings in a test equal or exceed the values shown

pregnancy may be forthcoming soon First, however, we must do more studies

A CURRENT ESTIMATE OF THE PREVALENCE OF DIABETES MELLITUS IN THE UNITED STATES

Quentin R Remein

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The size or importance of a disease as a public health problem is commonly determined by the number of persons who die of it, the number of persons who acquire it each year, the number of persons who have it, or the number of persons who become disabled because of it. Interrelationships among these statistics supply additional definitive information. Each of these measurements is deficient as a single gauge of the importance of a disease

This discussion is limited to the prevalence of diabetes, which is the number

concern about the number of cases of diabetes if it were not for the fact, for example, that diabetes is the eighth leading cause of death The longevity of diabetics is still considerably below that of the general population, ranging from 17 fewer years of life among diabetic 10-year-olds to almost 4 years less

from clinical observation of the day-to-day impact of diabetes on the lives of patients and their families.

Prevalence data will help focus the picture of diabetes as a public health problem From such data come estimates of needs for physicians, nurses, nutritionists, and other personnel in caring for diabetics, needs for insulin and other medical supplies and equipment, and better definition of extraordinary needs in times of national emergency, as well as in ordinary situations, and the rationale for allocation of research funds The estimate of unsuspected cases of diabetes unknown to patient or physician shows the need for case finding

The need for such prevalence data has not gone unnoticed among investigators in the medical field I shall not attempt to recount the studies con-

estimate based on adequate statistical methodology, therefore, any estimate of diabetes prevalence that could be presented is of necessity a crude working estimate based on scattered data and is verifiable only by comparison with local surveys and studies

One might ask why, considering its importance as a health problem, there has not been a definitive study of the prevalence of diabetes This same question can be posed for any disease because there has not been much practical

11. WILKERSON, H L C & Q R REMEIN 1957 Studies of abnormal carbohydrate metabolism in pregnancy the significance of impaired glucose tolerance Diabetes 6(4): 324–329
12 CARRINGTON, E R, H. S REARDON & C R SHUMAN. 1959 Recognition and management of problems associated with prediabetes during pregnancy J Am Med. Assoc 166 245–248.
13. JOSLIN, E, H ROOT, P. WHITE & A MARBLE. *Ibid* : 95.

is not independent of methodology, and we may expect to see development of methods for more accurate prevalence estimates at a reasonable cost.

About 14 years ago the Public Health Service, Washington, D. C , became aware of the public health implications of the diabetes problem in the United States and the possibility of applying to diabetes problems many of the public health techniques that had proved so successful in controlling the communicable diseases Early public health interest in diabetes led to investigation of the size of the problem and to estimates of prevalence The need for and interest in such data are no less acute today than they were fourteen years ago.

Historically, the prevalence estimates made by the Chronic Disease Program of the Public Health Service have originated from the well-known study of Wilkerson and Krall in Oxford, Mass , in 1946 and 1947.[3] In the course of this pioneer study of prevalence in a typical United States community, over 70 per cent of the population of the town was examined for diabetes, using history and blood and urine sugar studies A rate of 17 5 cases of diabetes per 1000 population was obtained A few years after this study started several large-scale case-finding programs were conducted in which there was no attempt to study a population sample On the basis of the Oxford study and [illegible]

[illegible]

TABLE 1
ESTIMATED PREVALENCE OF DIABETES IN THE UNITED STATES IN 1958

	Number	Rate per 1000 population
Unsuspected diabetes	1,400,000	8 1
Known diabetes	1,500,000	8 8
Total diabetes	2,900,000	16 9

May I point out that the estimate refers only to the noninstitutionalized population, it accepts the prevailing diagnosis of known cases as being correct, and is based on conservative diagnostic criteria used to determine the unknown and unsuspected cases in the population The Chronic Disease Program has endeavored to provide as reasonable and conservative an estimate as possible within the limitations of available data

The current estimate of the prevalence of diabetes in the United States, then, is 2 9 million cases Of that number, roughly 1 5 million cases are estimated to be known, and about 1 4 million are unsuspected cases (TABLE 1) These data produce rates of 16 9 per 1000 population in total, 8 8 known cases per 1000, and 8 1 unsuspected cases per 1000 population

development in the field of national prevalence data; however, I believe that major developments of this sort will take place in the next few years Some of the reasons for the current lack of definitive information on diabetes prevalence may help us to appreciate the problems involved:

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numbers of cases of diabetes that would meet even the most conservative diagnostic criteria. There are also many persons who would be called diabetics by some criteria but not by others, and there are even those referred to as prediabetics This rather necessary ambiguity is reflected in estimates of prevalence

In this connection it may readily be seen that changes in what is called diabetes will automatically produce parallel changes in prevalence estimates

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(2) Another reason for the lack of definitive information on disease prevalence is that the methodological problems of measurement were almost insurmountable until intensive study during the last five years brought forth some major breakthroughs Even now, however, this is a very difficult area.

Various means have been attempted to uncover the prevalence of diabetes as known to the people themselves and to their physicians The household survey on a sample basis is perhaps the most frequently used In addition to

members of any sample population selected for study have actually accepted the examination This is a serious source of bias Reasons for nonresponse

or in the next few years,
diabetes and other diseases is worth the high cost involved. The cost problem

prevalence that is known to the patient or physician A large-scale case-finding program may alter significantly the known case rate at the expense of the rate of unsuspected cases

The methods used in the surveys shown vary widely and are worthy of comment In the Oxford study previously referred to, histories were obtained on all persons examined, and the information was verified with the physician whenever a person said that he had diabetes [3] The New Market, Ont , Canada, study was conducted in similar fashion;[4] more than 80 per cent of the population was tested The data on Baltimore, Md , were obtained from the Study of the Prevalence of Chronic Illness conducted by the Commission on Chronic Illness [5] This study consisted basically of a household-interview survey of a random sample of the population followed by a "clinical evaluation" of a subsample of interviewees This clinical evaluation obtained a response rate of about 63 per cent. As stated in the Commission's report, in reference to it, "The diagnosis of diabetes was based on clinical judgment taking into consideration history, physical findings, blood sugar and urine sugar determinations "

TABLE 4
PREVALENCE OF UNSUSPECTED DIABETES IN SEVERAL STUDIES COMPARED WITH UNITED STATES ESTIMATE
(Rates per 1000 Population)

Area of survey or estimate	Crude rate	Adjusted rate*
Oxford, Mass	8 0	9 3
New Market, Ont	4 8	4 9
Baltimore, Md	15 5	13 3
United States prevalence estimate		8 1

* Adjusted according to the distribution of the 1957 United States population by race and broad age groups

The California, Hagerstown, Md , and Kit Carson County, Colo data were obtained by household-interview surveys of samples of the population [6-8] Diagnosis of diabetes as reported by the respondent was not verified

We see a wide range of rates in this group of studies The result of the California Health Survey stands out as exceptionally low The relationship between mortality and prevalence is by no means a sure one, but the mortality rate from diabetes in California is only two thirds that of the United States as a whole It is therefore conceivable that the prevalence of known diabetes is considerably less in California than in the nation as a whole The comparison between the current estimated national rate and the several studies is best shown by the race-age adjusted rates [illegible]

. . .

In comparing rates of unsuspected diabetes we are again limited to the few studies in which examinations of a sample of the population were actually undertaken, these are shown in TABLE 4 The current estimate for the United

To our knowledge, there have been few studies reported in which an examination of any kind was given to obtain an estimate of the total prevalence of diabetes While the criteria for diagnosis of previously unknown cases are not the same, the 3 studies shown in TABLE 2 provide some comparison with the Chronic Disease Program estimate The Canadian study[4] rate is considerably less than that for the two eastern United States studies[3,5] Both of the United States studies have considerably higher rates than the national prevalence estimate and were conducted in areas having high diabetes mortality rates From case-finding programs one also gets the impression that these areas have higher-than-average prevalence rates This fact, of course, is discernible in the comparison of these 2 rates to the national estimate While I am indebted to source material for the basic data on these various studies, the responsibility for rates and adjustments thereon is my own, and not that of my sources of data.

TABLE 2
DIABETES PREVALENCE IN STUDIES WITH PHYSICAL EXAMINATIONS COMPARED TO UNITED STATES ESTIMATE
(Rates per 1000 Population)

Area of survey or estimate	Year	Crude rate	Adjusted rate*
Oxford, Mass	1946–1947	17 5	21 2
New Market, Ont	1949	12 2	12 8
Baltimore, Md	1953–1955	26 7	23 0
United States prevalence estimate	1958		16 9

* Adjusted according to the distribution of the 1957 population of United States by race and broad age groups

TABLE 3
PREVALENCE OF KNOWN DIABETES IN SEVERAL STUDIES COMPARED WITH UNITED STATES ESTIMATE
(Rates per 1000 Population)

Area of survey or estimate	Year	Crude rate	Adjusted rate*
Oxford, Mass	1946–1957	8 0	11 9
New Market, Ont	1949	7 5	7 9
Baltimore, Md	1953–1955	11 3	9 7
California Health Survey	1954–1955	5 5	5 5
Hagerstown, Md	1955–1957	8 7	7.5
Kit Carson County, Colo	1957	9 8	9 0
United States prevalence estimate	1958		8 8

* Adjusted according to the distribution of the 1957 United States population by race and broad age groups

portant individual and public health problem and that large numbers of unsuspected cases of diabetes await detection and medical care.

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States is again lower than that of the Oxford and Baltimore studies, but higher than that for the Canadian study.

A second source of crude comparative data on unsuspected diabetes is found in the results of case-finding programs conducted in various areas The persons screened in these case-finding programs were not necessarily representative of the entire population in the area in which the programs were conducted To the extent that these programs were successful in attracting high-prevalence groups in the population, their results were also less representative of prevalence in the general population There have been 64 diabetes-screening programs with diagnostic information reported in the past several years to the Public Health Service[9] In the average program 6 new cases of diabetes were discovered per 1000 persons tested, but unfortunately there is no breakdown of the results of these programs by age of persons tested In most programs only a few persons under 25 years of age were tested It is known that the older population, particularly those aged 65 years or more, are poorly represented in such programs If the 2 under-represented groups, that is, the young very-low-prevalence group and the aged high-prevalence group, to a great

used diabetes-screening techniques have a sensitivity of about two thirds. Thus, in a random screening of persons not in a fasting state 2 of 3 persons

positive in a screening test such as the

level on venous blood or its equivalent

tics will be missed. These findings are

Therefore, the 6 cases per 1000 tested as found in case-finding programs repre-

national estimate

The future prospects are very good for more accurate and better-defined

difficult to evaluate the

of diabetes We believe it is a good, useful working estimate Regardless of the precise figures used, the conclusion is inescapable that diabetes is an im-

too rapid in any vessel to distinguish cellular detail At present there is no

wall, and sclera These differences can be enhanced by manipulating the illuminating source but, at best, healthy vessel walls are difficult to distinguish from the supporting parenchyma Therefore, in most instances the diameter that is measured is that of the flowing column of blood rather than that of the blood vessel This error is least for the capillaries and venules, but it may become appreciable in arterioles when there is an anemia because of the increase in the peripheral plasma layer. The column of blood, however, does delineate accurately the configuration of the wall that in health are cones for arterioles and venules, with the smallest diameter of the cone at the junction with the capillary The capillaries are cylinders. Cellular detail can be identified intravascularly whenever the velocity of flow decreases, which occurs in health when terminal arterioles constrict, when arteriovenous-anastomoses open (which decreases the flow rate distal to the shunt), and in short venules that connect adjacent venules and in which the pressures are nearly equal, which reduces or stops the flow in the connecting vessel Under the above

vessels It has been stated that reduction of the linear velocity of blood flow per se produces erythrocyte aggregation[2] This is not true in health. The blood flow through many areas of the body, especially in the liver, can stop for many minutes, and erythrocyte aggregation does not occur, however, they will form aggregates when the interfaces of the erythrocyte are abnormal The

and pressure, the normal number of intravascular cellular and plasma constituents, and a normal blood volume, a characteristic vessel pattern exists in the bulbar conjunctiva The ratio in diameter between corresponding segments of arterioles and venules is 1.1 75 to 2 0 The vessels are not tortuous, and the pattern of distribution is regular With a healthy intravascular and vascular physiology the connective tissue of the bulbar conjunctiva is transparent, permitting sharp focus of the circulating blood (this does not occur when there is a maintained reduction of blood flow through the microvascular system with associated exudation of plasma, whereupon the edema fluid causes increasing opacity of the connective tissue, with a concomitant decrease in the optical resolution). The morphology can be quantitatively determined with the use of a calibrated scale in the ocular of the microscope and a chronometer to measure the temporal events[3]

Recording the Dynamic Morphology

If the observations are to be meaningful, the method of recording assumes importance Difficulties exist. The microvascular physiologists have not

DIABETES MELLITUS AND THE LIVING MICROVASCULAR SYSTEM

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Can anything be learned about the mechanism that damages blood vessels in diabetes mellitus by studying the dynamic morphology of the superficial microvascular system in man? The current opinion in regard to the answer to this question will be derived through a review of (1) the preferential site for the study of the microvascular system, (2) the structures that can be visualized in this tissue, (3) the recording of the dynamic morphology, (4) the statistical validity of data derived from superficial microvascular systems, and (5) an analysis of the studies to date

Preferential Site for the Study of the Microvascular System in Man

The tissues that can be considered practical for study are the skin, the mucous surfaces, the bulbar conjunctiva, and the retina. Of these tissues the bulbar conjunctiva is preferred, because the blood can be followed with ease as it passes through the numerous arteriolar-capillary-venous channels. Good definition of the circulation is obtainable because the vessels are superficial, parallel to the surface, and embedded in transparent connective tissue, and contrast is enhanced by the white background (the sclera). Also, this tissue is constantly irrigated by normal physiological fluid Therefore, special preparation of the tissue is not required, and homeostasis is automatically maintained The result of the anatomical and physiological constituents permit

... Some of the inherent limitations in the study of the vascular and intravascular reactions of the retina at the microscopic level may be obviated or eliminated in the future by electronic image devices The mucous surfaces and skin are not suitable because of their special vascular anatomy,[1] their terminal vessels are at right angles to the surface It is difficult to differentiate not only the terminal portion of the arteriole but also the origin of the venule, since the capillary

lying keratinized epithelium transparent

Dynamic Morphology

When a microscope is focused on the bulbar conjunctiva, the component most readily visualized is the circulating blood With the magnifications that are used most frequently (48 to 150×) the velocity of blood flow in health is

in the capillaries and venules in the bulbar conjunctiva is not necessarily a statistically valid sample. The sample will reflect statistical validity of connective tissue blood vessels similar to those that are not the site of active special metabolic processes of physiology or disease. For example, the physical composition of the blood is altered in capillaries and in the immediate postcapillary venules in tissues undergoing active metabolic processes such as muscular contraction or processes of localized disease These processes can cause localized hemoconcentration or the formation of cellular aggregates that may not be reflected in the bulbar conjunctiva due to the dilution of these abnormalities by nonaffected blood flowing through adjacent tissues and diluted in the heart and lung by mixing The arterioles in the bulbar conjunctiva or any other arteriole in the body contain a statistically valid sample of blood, while the blood in the capillaries and venules are statistically valid for the [illegible]

Data Derived from the Bulbar Conjunctiva

To be able to evaluate the current studies it is worthwhile to review briefly the history of the microvascular system in man[3] The study began more than one hundred years ago. Many of the early investigators noted cellular aggregation and abnormalities of vessel walls Reports of microaneurysm have been described in diabetics for more than 50 years. Similar pathology was also observed in other diseases Often the reports were conflicting or difficult to interpret, which led to a nihilistic attitude in regard to the usefulness of the bulbar conjunctiva to assess vascular damage[11] This attitude has not been dispelled entirely[12-14] In the past this attitude was justified, since the knowledge of what constituted the healthy microvascular system was meager In respect to the dynamic morphology of this system, such an attitude can be maintained no longer Relevant data for the system exist that have been ob- [illegible]

ferences of opinion are not due to inaccurate data, but to inaccurate interpretation of them There is a failure to understand clearly that a description of this system as pathological per se does not necessarily imply significance in respect to an alteration of function of the entire organism that can be detected [illegible]

define in general an abnormal microvascular system and indicate some of the mechanisms that produce the abnormality. The discussion that follows is based on data derived by many methods and includes experimental animals where better optical resolution could be obtained and higher magnification (to 1270×) could be used[3, 9, 27, 28]

By definition, the healthy microcirculation becomes pathological whenever

the stranding of these cells in small blood vessels and presumably (indicated by direct observation in experimental animals) by phagocytosis in the liver

In summary, the abnormal circulation is defined as the formation of aggregates by the formed elements of the circulating blood and/or an increase in the plasma viscosity that results in making the blood more difficult to circulate *through the microvascular system*, which can result in concomitant pathology of the vessel walls and a reduction in the circulating blood volume through thromboembolism, phagocytosis, and loss of plasma fluid

A variety of abnormal processes can influence the microvascular system and, due to the limited number of responses of this system, morphologic similarities *will exist* *The differences of effect on the system* will be determined by the metabolic disturbances of the disease For example, cellular aggregation is the result of altered metabolism of the host due to a variety of noxious stimuli such as tissue trauma, antigen-antibody reactions, or bacterial or viral infections Some of the factors that influence erythrocyte aggregation are the chemical nature of the stimulus (*physical trauma produces large rigid* (20 to 40 μ) aggregates in contradistinction to a tuberculous infection where the aggregates may be small (10 to 13 μ) and soft), the duration of the stimulus (in trauma it is short and not repetitive while, in a tuberculous process, it can be active for months or years), the volume of tissue involved with patent blood flow through the infected area (in local trauma a very small volume of blood is involved while, in an extensive bacterial pneumonia, the volume is large), and the physiological status of the host (the aggregation of erythrocytes is less in malnutrition) Little work has been done to isolate the specific chemical inducers of such aggregation [8, 29]

The above review illustrates some of the abnormal stimuli that can exist in disease, resulting in the production of intravascular cellular aggregation that produces pathology of vessel walls indicated by spasm, dilatation, constriction, or aneurysms In general, each disease is limited by the damage it causes, which cannot be compensated for by the defense mechanisms of the host Therefore, in a general manner, the dynamic intravascular and vascular reactions analyzed over the entire period of the disease will follow a rather consistent pattern, this has been documented [2, 7, 8] However, no visible intravascular or vascular reaction per se is pathognomic, so that a diagnosis can be made only by observing the microvascular system

Biomicroscopy of the human microvascular system of the bulbar conjunctiva began with Coccius [30] Among the earlier investigators were Donders,[31] who recognized erythrocyte aggregation, Bajardi,[32] who was interested primarily in reaction of the walls of blood vessels to drugs, Schleich,[33] who stressed the importance of measuring blood flow, Reimar,[34] who recognized that clumps of cells will sediment as flow decreases and described the effect of cellular aggregates on flow, but who recognized that these were important only when thrombosis occurred, and Luedde,[34] who clearly distinguished the difference of flow characteristics between arterioles and venules and biopsied the conjunctiva The early reports of diabetes are those of Streiff,[35] who described granular flow and thrombi, Zeller,[36] who observed microaneurysms and invented a method for measuring the rate of blood flow, and Cesari,[36a] who correlated the changes

an aggregate is present (erythrocyte, leukocyte, or platelet) and/or the viscosity of the plasma increases.[3] The aggregate, which is larger than the other cellular elements, changes the character of the flow. Independent or associated with aggregate formation is the adherence of leukocytes and/or platelets to the walls of postcapillary venules. Whenever the linear velocity of the flow is reduced, the first vessel walls that indicate dynamic morphologic abnormality are the postcapillary venules where hemoconcentration and dilatation occurs.

cytes are more commonly involved than the other formed elements. Therefore, with the continuation of the process that produced the formation of an aggregate, the majority of the circulating cells may become involved. At first the forces that bind the cells into aggregates will be weak, and the process occurs only in stationary or very slowly flowing blood, but finally the strength of the forces may be so strong that the aggregates are not fragmented in the arterial circulation. As a result of the increase in size and rigidity of the aggregates the velocity of blood flow decreases correspondingly.

The change from the healthy circulation is the degree of abnormality or "sludge" present at that moment.[3] With multiple observations it becomes possible to determine, not only the extent, but also the rate of the intravascular and vascular changes.

When an abnormal circulation is present:

(1) Either erythrocytes, platelets or leukocytes, or a mixture of such cells form aggregates.

(2) Leukocytes and platelets adhere to the endothelium of systemic blood vessels and, under special conditions, erythrocytes adhere to the leukocytes that line the vessel wall.

(3) In the presence of an increasing number of aggregates the normal laminar blood flow changes. At first there is an increase in the thickness of each lamina, followed by mixed flow that produces gross turbulences, finally, plug flow occurs where the aggregate is as wide as the vessel.

(4) The disturbance in the laminar flow produced by the aggregates results in a decreased velocity of blood flow. With the reduction in flow and the presence of aggregates, the homogeneous appearance of the healthy, flowing column of blood disappears, and the cellular aggregates become increasingly visible and sediment.

(5) Due to the decreased blood flow excessive plasma fluid is lost through the walls of venules.

As a result of such a circulation:

(6) The walls of blood vessels fail to receive adequate nourishment and the abluent properties of normal flow are altered, which results in a loss of their normal cone-shaped configuration.

(7) The walls of venules become increasingly permeable to the plasma that produced hemoconcentration, with associated local or general dilatation and sacculation and edema of the adjacent tissue.

(8) Finally, the number of circulating erythrocytes begin to decrease due to

extent, in the pituitary, kidney, and pancreas, but such studies have not been made

The question was proposed whether anything could be learned about the mechanism that damages blood vessels in diabetes mellitus by studying the dynamic morphology of the superficial vascular system in man The question may be answered affirmatively because the blood flowing through the arterioles represents a statistically valid sample of all the arterial blood. The responses of the vessels in the bulbar conjunctiva represent those of connective tissue Therefore, it is not possible from these data to predict with certainty how the microvascular systems of the pancreas, liver, pituitary, or kidney will respond It is recommended that experimental animals be used to elucidate the responses of these organs and to obtain further data in man from the peripheral circulation

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seen in the circulation of the skin and conjunctiva and stressed the fact that these results were significant also for kidney and brain (also see Muller 1937–1939).[1]

The influence of intravascular erythrocyte aggregation on the microvascular system was stressed by the reports of Knisely *et al.*, which were followed by reports from other laboratories.[38] In 1951 Ditzel[37] began to report data obtained in patients with diabetes mellitus, using the bulbar conjunctiva method described by Knisely and Bloch Ditzel and his associates have studied diabetic children,[39] normotensive diabetic adults,[40] and pregnant diabetics,[41] and have found that the vascular pattern is characterized by tortuosities and elongation of the capillaries, narrowing of the arterioles, dilatation of venules, and perivascular exudation leading to "hyaline" infiltration These changes were associated with varying degrees of erythrocyte aggregation Ditzel and his co-workers concluded that the venular distension and increased permeability are often maintained for long periods and, while these conditions are associated with cellular aggregation,

is minimal The abnorm,

the diabetes, and these are

and his co-workers are more than a corroboration of the earlier data for this disease, since they have used better criteria and better subjects. The finding of a specific reaction in the microvascular system in diabetes is not surprising, since "specific" patterns of reactions have been found in poliomyelitis,[7] myocardial infarction,[8] and tuberculosis[3] In these diseases similarities and differences exist in the reaction of the microvascular system This seeming paradox is explainable by the fact that only a few morphologic criteria are

omitant

It may

and differences in the response of the bulbar conjunctiva to chronic reduction in blood flow to obtain an insight into the mechanism of how these diseases damage the vascular system. The studies of Ditzel *et al* [37, 39-41] are commendable because they provide additional

e studies for a longer

e are to be elucidated.

more studies required and for a longer period of time, but they should include quantitative results so that it will be possible to compare the data of different investigators

If the morphologic mechanism of how diabetes affects the vascular system is to be known, it is necessary to study other vascular beds. The study of the retinal vessels is curtailed by the optical limitation of magnification, which is difficult to overcome It may be possible to determine changes in the walls of venules, blood flow, and parenchyma with the present optics by using the technique of scanning microspectrophotometry.[42-45] This method shows great promise, but it is not yet perfected and, even if the promise is fulfilled, this technique will be insufficient. It is necessary to turn to experimental animals so that one may study the sites where the metabolic disturbances are originating or producing their major disturbances Methods and information exist that make it possible to study the microvascular system in the liver and, to a lesser

DIABETIC NEUROPATHY: EVALUATION OF FACTORS IN ONSET

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Although diabetic neuropathy has long been recognized, there are still many areas of inadequate knowledge and uncertainty. The diagnosis itself presents

diabetic control Among the authorities subscribing to this viewpoint are Joslin,[3] Duncan,[4] Rundles,[5] Goodman,[6] and Martin[7]

are manifest in several directions and include the following six points (1) neuropathy may occur during good control; (2) there may be a simultaneous onset of neuropathy and the symptoms of uncontrolled glycosuria, (3) the neuropathy is unrelated to the duration or severity of the diabetes, (4) neuropathy may be the initial clinical manifestation of diabetes, unattended by

and in these instances, a relatively constant latent period exists

(1) *Neuropathy during good control* There is not infrequent reference in the literature to the occurrence of neuropathy in this clinical situation The areflexia, pain, and paresthesia of diabetes commonly occurred in patients with diabetes under thorough control in the series of Woltman and Wilder[8] Similar observations were made in three cases of neurogenic bladder[9] and in a series of patients under close supervision, good care, and good control[10] Charcot's joints have been reported in well-controlled diabetes[11] Other authors alluding to this set of circumstances include Broch and Klovstad,[12] Garland and Taverner,[13] De Jong,[14] and Jordan[15] The last author stated that, since neuritis commonly occurs more often in the older person with mild diabetes than in the young one with severe diabetes, diabetic neuritis is not the result of hyperglycemia alone, and one must seek a more subtle factor[16]

(2) *Simultaneous onset of neuropathy and diabetes* This situation is well documented The neuritic symptoms dominate the clinical picture, and the diabetic symptoms are elicited usually by a careful, routine history. This sequence of events was reported by Root and Rogers,[17] Bailey,[18] Epstein,[19] Boeck,[20] Broch and Klovstad,[12] and Jordan.[15] In our patients with this type of onset, no previous evidence or history of glycosuria could be demonstrated

(3) *Neuropathy unrelated to duration or severity.* If poor control is the most important aspect in the pathogenesis of neuropathy, then severe diabetics (as measured by insulin requirement) and those with diabetes of long duration

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surgery, amputations, cerebral vascular accidents, diabetic coma, barbiturate coma, acute myocardial *infarction*, acute *infections*, corticosteroid therapy, trauma, and the institution of control of the diabetes by diet, insulin, or tolbutamide

Another characteristic of this group of patients is the presence of a fairly constant time interval or latent period This latent period averages about 18 days, with a range from 7 to 30 days, its significance will be referred to later

TABLE 1

Stress factor	Latent interval (days)
Surgery prostate	24
Surgery prostate	29
Surgery amputation	10
Surgery abdominal	10
Infection	14
Infection	21
Myocardial infarction	20
Myocardial infarction	24
Barbiturate coma	7
Corticosteroid therapy	23
Cerebral vascular accident	30
Control with insulin	7
Control with insulin	28
Control with insulin	14
Control with insulin	20
Control with insulin	21
Control with insulin	15
Control with tolbutamide	14
Control with tolbutamide	21
Control with tolbutamide	28
Average	18 5

Table showing the latent interval of onset of neuropathy following the various stresses in 20 patients

Discussion

That prolonged poor diabetic control may possibly lead to neuropathy is not denied In addition, this paper is not meant to minimize the importance of good control in diabetes nor to enter into the controversial aspects of the merits of maintaining normoglycemia and aglycosuria in the diabetic patient, rather, the conclusions rest on the premise that neuropathy can and does occur independently of the presence, degree, or duration of hyperglycemia and glycosuria

There has been an *increasing* awareness that diabetes is a complex, generalized, fundamental disease process of which the carbohydrate metabolic disorder represents a single facet Other facets include pregnancy, microangiopathy (comprising retinopathy and nephropathy), and arteriosclerotic involvement of the larger vessels. All of the above may occur as the initial *clinical manifestation of diabetes.* Dry and Hines[34] felt that it would be correct to regard the vascular problem as a manifestation of an abiotrophy affecting the insulin-producing tissues and the vascular system. Recognition of this

should have the highest incidence of neuropathy. In our own experience there is no correlation between neuropathy and duration or severity of diabetes. Similar observations have been made by Jordan[15] Rudy[21] and Martin[22] [illegible]

[illegible]

[illegible] These cases can hardly be ascribed to a prolonged period of poor diabetic control. The diabetes in these cases is diagnosed by finding an abnormal glucose tolerance test, carried out because of suggestive or compatible neurological syndromes and/or the presence of a family history of diabetes. Continued follow-up of these patients indicates that the overt diabetes makes itself manifest

Similar observations have been recorded by Jordan,[16] Andrews,[23] Bailey,[18] Garland,[26] Sprague,[27] Muri,[28] Lincoff and Cogan,[29] and Broch and Klovstad[12] My personal experience, as previously published, is in complete accord with this concept

tio [illegible]

tio [illegible] neuropathy is a concomitant and not a complication of diabetes; this point will be discussed later

(5) *The paradoxical precipitation of neuropathy following the institution of diabetic control.* This situation is incompatible with the postulate that the condition is due to a prolonged period of poor control This observation has been sporadically reported in the literature[5, 21, 27] In a study directed toward this set of circumstances, I have witnessed this phenomenon not infrequently in patients controlled with insulin,[30] as well as in those controlled with tolbutamide.[31]

The means whereby control is achieved are unrelated to the sequence of events, since similar phenomena are observed with diet alone, with insulin, or with tolbutamide. The neuropathy in this category follows a fairly constant latent period of 2 to 3 weeks of good control A suggested explanation is that the sudden change in homeostasis results in a physiological aberration simulating a stress situation Since the neurological syndromes [illegible]

[illegible] from any intrinsic noxious role.

(6) *Stress situations leading to neuropathy* Occurring incidentally in the course of diabetes such situations may lead to neuropathy independent of the state of diabetic control[32]

Several authors have included this type of situation in their series including Rundles,[5] De Jong,[14] Rudy,[21] Epstein,[19] and Springer and Hymes[32] I have observed 20 cases wherein the neuropathy followed the incidental occurrence of stress situations (TABLE I).

The resulting neuropathies are entirely characteristic of the recognized syndromes of neurological involvement as they occur in diabetes, and their clinical representations are independent of the nature of the stress. These facts indicate that the neuropathies are definitely part of the diabetic state The stress factors that I have observed include abdominal surgery, prostatic

constant latent time interval suggests the presence of an operative toxic or metabolic factor in this category.

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approach to diabetes has led to the performance of glucose tolerance tests and the concept of the existence of a prediabetic state.[35, 36] Root *et al.*[37] felt that diabetes mellitus is hereditary, but may remain latent until some stress brings it forth This hypothesis carries with it a specific vulnerability of the central nervous and vascular systems. Thus, long before hyperglycemia and glycosuria are present, the patient may manifest other defective states.

From this point of view neuropathy emerges as an integral part of the variegated syndrome of diabetes mellitus It is a concomitant, not a complication, it becomes a fundamental component of a multifaceted crystal and not an end result of failure of proper clinical management of disordered carbohydrate metabolism

From the etiological consideration, no known factors have as yet been determined In view of the varying backgrounds preceding the onset of neuropathy it would seem reasonable to conclude that there are probably several such factors However, in those cases where the neuropathy follows stress situations, the evidence points to the presence of an operative toxic or metabolic factor This assumption is based on the presence of a fairly constant time interval that is independent of the precipitating factor. It is directly comparable to the latent period of many known inciting factors that lead to various types of neuritis These include postinoculation neuritis,[38] postvaccinal neuritis,[39] abortifacients,[40] arsenic,[41] and neuritis complicating penicillin therapy[42] This parallel adds to the significance of our observations and suggests the general category of a sensitization phenomenon mediated via a toxic or metabolic factor that produces the cause-effect relationship.

It is worthy of comparative note that toxic drug manifestations resulting in intrahepatic cholestasis without neural involvement also have a similar latent period These include arsphenamine,[43] thiouracil,[44] chlorpromazine,[45] chlorpropamide (unpublished observation), and metahexamide (personal communication from H Dolger)

The presence of a latent time interval strengthens the validity of the contention that the stress factors are related to the neuropathy and are not a coincidental association The fact that comparable situations with known toxic factors act in like fashion and express themselves similarly tends to support and corroborate this hypothesis

Summary

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development of neuropathy

Diabetic neuropathy should be regarded as a concomitant feature of the syndrome of diabetes mellitus rather than as a complication of the disease

Awareness of the occurrence of neuropathy as the initial clinical manifestation of diabetes may help solve some obscure clinical problems.

The diverse character of the factors preceding the onset of diabetic neuropathy suggests that there may be several etiological determinants. However, the sequence of neuropathy following stress situations after a relatively

ACCEPTABILITY OF DIABETICS FOR LIFE INSURANCE

Paul S. Entmacher

Metropolitan Life Insurance Company, New York, N Y

Only about 30 per cent of persons with a known history of diabetes applying to the Metropolitan Life Insurance Company for life insurance are found to be acceptable, according to a recently [illegible] experience. About two thirds of [illegible] use the offer, thus [illegible] diabetic applicants who even- [illegible] policies A study in 1949 of its diabetic applicants [illegible] other insurance company showed an acceptance rate of about 40 per cent,[1] and a panel discussion in 1953[2] by medical directors of several insurance companies showed a range of about 30 to 40 per cent. In the past decade, therefore, despite our ever-increasing knowledge of the disease, the diabetic population has shown little improvement from an insurance-underwriting viewpoint Why are 60 to 70 per cent of the diabetic applicants for insurance still found to be unacceptable for life insurance? What yardsticks are the insurance companies using in evaluating them?

Before the era of insulin therapy and for many [illegible] insulin was discovered all diabetics were [illegible] adually, however, [illegible] isure diabetics on [illegible] this lead, and today [illegible] issues substandard-risk life insurance offers some type of coverage to a selected group of diabetics It is an accepted fact, however, that even the most favorable group of diabetics cannot be considered for standard-risk insurance because, although their mortality rate has diminished, with a corresponding increase in life expectancy, it still is not as good as that of the general population In general, insurance companies will decline applicants for whom the expected mortality is greater than 4 to 5 times that of standard risks Substandard-risk insurance will be offered to the groups that have a mortality experience falling between the standard mortality limit and that for the declined group, the extra premium varying according to the amount of excess mortality A study of the experience of the Joslin Clinic, Boston, Mass, for the period 1947 to 1951 on patients first [illegible] in the years 1930 through 1951[3] [illegible] nting [illegible] population, [illegible] diabetics was almost 6 times greater [illegible] general population At age 30 it was about 10 times greater, at age 40 about 4 times greater, at age 50 almost 3 times greater, and at age 60 about 2½ times greater It is apparent, then, that diabetics decidedly fall into the substandard and decline [illegible] ically, all diabetics might [illegible] rance were devised [illegible] ated, but this idea

[illegible] lar [illegible] diabetics.

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tion of the enzymatic test, which is specific for glucose, is not likely to alter this Prior to the introduction of readily available commercial testing materials of this type, the Metropolitan Life Insurance Company was using the enzyme test in its biochemical laboratory and had demonstrated its accuracy conclusively[10] At the same time that this test was being evaluated, a study was carried out to determine if the results of a glucose tolerance test, usually performed on a different day, could be correlated
tal amount of glycosuria[11] The results were
rmed the impression
cose tolerance
Metropolitan
blood sugar test
whether or not the applicant is a
these results have a limited value An insurance company,

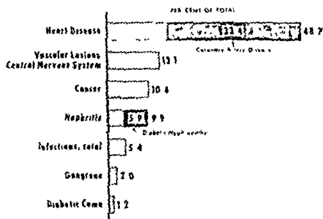


FIGURE 2 Selected causes of death among diabetic patients of the Joslin Clinic in the years 1950 to 1956

by the nature of its business, can obtain only one test in almost all instances, and it has no control over the type of dietary intake of the applicant during the days preceding the test Apart from this, the companies differ on test procedures and their interpretation some companies use fasting blood sugars, others postprandial blood sugars, and still others use various interval values after glucose ingestion The latter approach was adopted by the Metropolitan Life Insurance Company after a review (unpublished data) of two-hour glucose tolerance tests showed that the number of cases was so small where the fasting or half-hour blood sugar levels altered the interpretation of the test that it was deemed unnecessary to obtain the first two specimens. Granting, however, that variability in the type of test used and in the interpretation of the test exists, the question arises of how to handle the applicant who has a definitely abnormal level, say in the range of 130 to 150 mg per cent on the 2-hour specimen, but who does not appear clinically to be a diabetic. Different companies have their own individual approaches to the problem and, until more extensive

The over-all death rate from cardiovascular-renal disease is 2½ times greater in diabetics as compared to the general population. Analysis of the causes of death in recent years among diabetic patients,[3] as illustrated in FIGURE 2, shows that the diseases of the cardiovascular-renal system accounted for about three fourths of all deaths. Heart disease alone was responsible for nearly one half the total, and 2 of every 3 heart disease deaths were due to coronary artery disease. Next in order were cerebral vascular lesions, cancer, and renal disease. There is still some excess mortality among diabetics from infections, whereas death from diabetic coma and gangrene has decreased progressively.

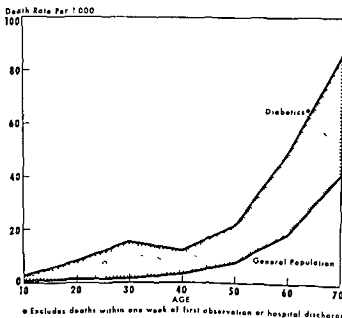


FIGURE 1. Death rate among diabetics and in the general population. The diabetic rate is that of patients of the Joslin Clinic in the years 1947 to 1951, the general rate is that of the white population of the United States in the years 1949 to 1951.

From an insurance-evaluation standpoint, there are two main groups to be considered: (1) persons with glycosuria, but without a specific history of diabetes, and (2) known diabetics. Concerning the first group, even before large-scale industrial health programs were begun, urine examinations in connection with life insurance probably represented the largest number of such examinations done on apparently healthy people. The discovery of numerous diabetics resulting from this has for many years been a major contribution of the life insurance industry to the field of diabetes detection.

Many studies have been made on the mortality of life insurance applicants with glycosuria.[4-8] It was found that those applicants who were limited to substandard insurance because of glycosuria had a mortality significantly above normal.[4-6] The amount of glycosuria, however, was not found to be a reliable index on which to base a substandard rating,[6, 9] and even the recent introduc-

cases examined in the years 1940 to 1949 and traced to 1955 These data also tend to show an increasing mortality with duration of disease that is most apparen
of disea
and deals with patients aged

TABLE 1
PER CENT RATIO OF ACTUAL TO EXPECTED DEATHS AMONG DIABETIC PATIENTS ACCORDING TO DURATION AT FIRST OBSERVATION
Cases With Insignificant or No Impairments

Age at observation (years)	Duration of diabetes at first observation	
	Less than 5 years (percentages)	5 Years or more (percentages)
25 to 34	329*	—
35 to 44	301	351
45 to 54	227	375
55 to 64	187	264

Experience of Joslin Clinic in 1939 to 1947 on patients first seen in 1930 to 1947 Expected deaths based upon Metropolitan Life mortality experience on standard risks.
* Based upon 7 deaths or less.

TABLE 2
DEATH RATES PER 1000 AMONG DIABETIC PATIENTS AGED 45 TO 59 YEARS AT FIRST OBSERVATION
Cases First Seen Less Than 1 Year and 5 Years or More After Onset

Duration from first observation	Duration of diabetes at first observation	
	Less than 1 year	5 Years or more
1 to 5 years	19 6	38 6
6 to 10 years	32 8	62 7
11 to 15 years	30 1	58 1

Experience of Joslin Clinic on cases examined in the years 1940 to 1949 and traced to 1955

Third, control of the disease must be evaluated In this regard the insurance

[illegible]

disease or impairment, the total character, temperament, and surroundings of the diabetic must be taken into consideration when considering him for insurance It helps if he has a good understanding of his disease and wants to

[illegible]

is also important. The experience of the Joslin group has for many years in-

mortality studies are carried out, this will continue to be an indefinite group. The mortality study carried out by Jimenis *et al.* on applicants who were given glucose tolerance tests did seem to indicate, however, that this group as a whole had a better mortality than the known diabetics. It also seemed to indicate that the mortality experience was less favorable in the group that had a grossly abnormal test as opposed to the group with a borderline or slightly abnormal response. Based on this experience, our approach at the Metropolitan Life Insurance Company has been to offer various substandard classifications according to the degree of abnormal response on the glucose tolerance test. We know that some persons in this group are diabetics but, within our pattern of operation, we cannot go ahead to establish the diagnosis. We are therefore dealing with the probabilities of a lesser or greater proportion of these applicants being diabetics, and our graded ratings are related to that. Analysis of mortality when we have a sufficiently large experience will indicate whether the ratings are correct

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Juvenile diabetics are still not con-
[illegible] c mortality statistics to support this contention,[2, 12, 13] and most companies will not issue insurance to diabetics whose onset of disease occurs prior to age 15. At the other end of the spectrum an upper age limit of 55 is often set, but some companies have an upper age limit of 60, or even higher. This aspect of the problem will undoubtedly undergo re-evaluation in the near future because of the separation of diabetics into at least two major groups. One group has its onset of disease earlier in life, is usually insulin-deficient, and in many cases has a "brittle" type of diabetes The second and major group consists mostly of obese persons with onset of disease in middle or later life. The difference between the two groups has been accentuated recently by the type of response to oral hypoglycemic agents

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entry to the clinic and duration of disease at first observation The results are expressed in ratio percentage of actual deaths in the diabetic group to expected deaths in a standard population group. The company's contemporaneous mortality experience on standard risks was used as the basis of comparison in formulating the ratio. Bearing in mind that the limit of insurability for any impairment is now a c

ratio of 100 per cent),

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lower mortality [illegible] esents the death rate per 1000 in the general diabetic population as seen at the Joslin Clinic on

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impairments at first observation, as compared to the death rate in diabetics with insignificant impairments or none

Taking all these factors into consideration, what has been the actual experience with diabetics who have been screened carefully and issued insurance? It is still too early to say accurately because of the limited material available to date, but preliminary studies[15, 16] show that, even in this carefully selected

...

uation of diabetics have a sound basis This is still an early experience, however, and because of the nature of the disease it is to be anticipated that the late experience will be less favorable. It is conceivable that some test or refined set of criteria may evolve that will predict prognosis with sufficient accuracy to allow an almost standard classification of certain diabetics At any rate, it is hoped, that after more experience has been accumulated and analyzed, more diabetics will be considered insurable and broad subgroups will be delineated, so that the better risks can be given more favorable ratings

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dicated that control is one of the most important factors, if not the one most important factor, determining when vascular complications will have their onset. Certainly, a great deal of stress is placed on control when an insurance underwriter attempts to assess the risk for a particular diabetic applicant.

Fourth, an attempt is made to estimate the severity of the disease. Some companies use insulin dosage as an indicator of severity, and 75 to 100 units of insulin daily is frequently set as a top limit for insurability. While insulin dosage is important, it is not a really good indicator of severity, and is not accepted as a hard-and-fast criterion of insurability. The recent advent of

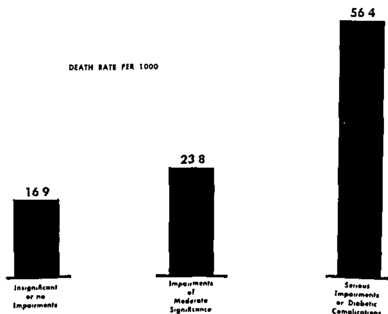


FIGURE 3. Death rate per 1000 of diabetic patients with and without significant impairments at first observation. These patients, aged between 15 and 64 years, were first seen in the years 1930 to 1945 (analysis made by the Metropolitan Life Insurance Company of Joslin Clinic records)

oral hypoglycemic agents for the treatment of diabetes has been watched with great interest by insurance companies and may influence underwriting practices

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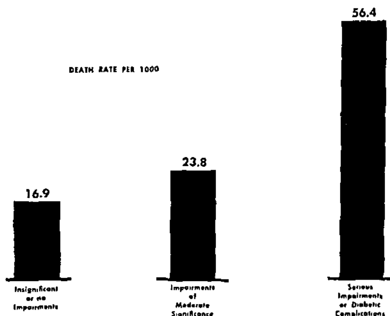


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oral hypoglycemic agents for the treatment of diabetes has been watched with great interest by insurance companies and may influence underwriting practices in the future. A good indicator of severity is the presence of other impairments

that even moderate amounts of albuminuria, hypertension, or both, had a markedly adverse effect on mortality, whereas a moderate degree of obesity had much less of an influence. The marked effect that small amounts of albuminuria and/or hypertension had on the mortality would seem to indicate either that, without knowing it, diabetics who already had renal involvement

may produce prolonged or permanent disability, which may be expensive to management

(5) *Increased insurance costs* This is frequently given as an objection to employing diabetics However this reason is reported to be unfounded, because group insurance [illegible] inclusion of diabetics

Some of these obje[illegible]
placement and periodic [illegible]
and proper *job placement*

In a study of the diabetic [illegible]
the results of a questionnaire submitted [illegible]
employee[illegible] [illegible] of diabetes among 280,602 employ[illegible]
39 compa[illegible] [illegible] 63 companies
employed [illegible]
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and 6 had no set policy Thirty-eight comp[illegible]

TABLE 1*

DATA CONCERNING 40 KNOWN DIABETICS IN AN INDUSTRIAL POPULATION OF 3508
(Incidence of 1 1 Per Cent)

	Diabetics	All employees
Age	32 to 68 years	19 to 65 years
Average	54 5 years	46 1 years

Family physician —17
Unknown — 5

* Data from [illegible] 1953 [illegible]

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of work afte[illegible]
TABLE 1 [illegible]
5 years These 40 diabe[illegible]
industrial population of 3508 persons employed by [illegible]
tem

The age of the diabetics varied from 32 to 68 years, and averaged 54 5 years The age of all employees varied from 19 to 65 years, and averaged 46 1 years Four persons had diabetes of several years' duration prior to employment The duration of em[illegible]ment after discovery of diabetes averaged 3 9 years, [illegible]

EMPLOYABILITY OF THE DIABETIC

Harold Brandaleone
New York University College of Medicine, New York, N Y.

The most frequent question the newly detected adult diabetic requests of his physician is, "Will I be able to continue in my job?" The male patient wishes to know if the disease will interfere with his ability to work; the female becomes concerned about her job or her household responsibilities

The physician must be able to answer these questions with accuracy and understanding. Usually the answers are simple, especially in the diabetic who is controlled by diet or by diet and an oral preparation. The patient who requires insulin for his control may present an employability problem if hypoglycemic reactions tend to endanger his own life or the lives of others

The diabetic requiring insulin should never pilot an airplane or operate a [illegible] to work near an excavation or [illegible] hat he is capable of supporting his family or of performing her usual household responsibilities. The diabetic child must be instructed early that he cannot be a jet pilot, but that he can do almost anything else The best place for such instruction is the diabetic clinic or summer camp, where lectures on suitable employment are prescribed as part of an educational program

Various reasons are given by employers for rejecting diabetics for employment I shall demonstrate on the basis of surveys made in industry that education can remove most of the objections to the employment of the diabetic. [illegible] or employment ar[illegible] compensation costs, [illegible] costs

(1) *Insulin shock* Sudden unconsciousness may endanger the life of an employee, as well as the lives of others For that reason proper job placement of the diabetic is important For example, the Interstate Commerce Commission, Washington, D C, records the case of a diabetic truck driver who required insulin He neglected to inform his employer of his disease, and was not required to submit to a physical examination. He was employed as the driver of a large trailer truck operating across the country One day, while driving on a mountain road, he lost consciousness, and his truck went out of control and struck several parked automobiles in a small town This accident cost two lives, many injuries, and a great deal of property damage

(2) *Prolonged absenteeism* Employers fear the high cost of prolonged absenteeism because of its effect on production schedules, overtime costs, and disability insurance A diabetic may develop tuberculosis or vascular disease and may be required to be absent for long periods of time.

(3) *Increased compensation costs* Employers realize the possibility of prolonged disability following an industrial accident. A minor injury to a lower extremity may produce ulceration, gangrene, and possible amputation

(4) *Complications of diabetes* Complications, especially vascular disease,

the tuberculosis and not because of his diabetes. The other was absent for 146 *of the 273* days because of an ulcer on the foot *While these* 2 patients represent only 6 per cent of the diabetics, they were responsible for 61 per cent of

[illegible]

absentee rate as for the nondiabetic group

(3) *Caliber of work as defined by accident rate* An effort was made to compare the caliber of work, as measured by the accident rate, performed by diabetic and nondiabetic bus operators

Thirteen of the 17 *diabetic* bus operators drove in routes of the city of New York The statistics for the diabetics as compared to *those for* the total group are shown in TABLE 3

Although, from a statistical viewpoint, the number *in the diabetic* group is deemed insufficient to permit a conclusion, the data for the diabetic group seem

TABLE 3*

NUMBER OF ACCIDENTS PER MAN IN 2-YEAR PERIOD FROM JULY 1, 1950 TO JUNE 30, 1952

	300 non diabetic operators	Diabetic operators	Number of cases
First 2 years of driving	5 8		0
Third year of driving	5 2		0
Fourth year of driving	5 3	5.6	6
Fifth year of driving	2 7	3 6	5
Sixth year of driving	3 3		
Seventh year of driving	3 2	3 0	2
			13

* Data from Brandaleone and Friedman, 1953 [1]

not far removed from those for the nondiabetics Furthermore, both sets seem to show a sharp decrease in accidents after the fourth year of operation

Another study was made by the Committee of Employment of the American Diabetes Association, Inc , New York, N Y [2] In response to questionnaires, 12[illegible]

dia[illegible]

in[illegible]

Eighty-eight companies [illegible]

betics, while 31 (25 per cent) do not, 8 companies (6 per cent) did not answer

Of the 31 companies that do not employ known diabetics, 17 based their [illegible] ", 4 on "Statistics [illegible] gave more than 1

Companies employing known diabetics rated their experience as to rehabil-

Eleven employees took insulin regularly. Sixteen took no insulin, and 6 took insulin irregularly In the remaining cases no data were available

Applicants with diabetes requiring treatment with insulin were not rejected except for the position of bus operator For such persons, employment in other work was recommended

Diabetic control was classified in four categories. (1) good, (2) fair, (3) poor, and (4) unknown A person was considered under good control when he maintained his body weight and had minimal glycosuria and no acetonuria. Fair control was signified by moderate to severe glycosuria, maintenance of body weight, and absence of acetonuria Control was considered poor when the person lost weight and had severe glycosuria and intermittent acetonuria

Seven patients maintained good control, 6 were fair, and 3 were poor; in 24 the status was undetermined. Seven of these 24 are no longer with the company The remaining 17 (43 per cent of the total number of diabetics) did not take advantage of the opportunity to cooperate with the medical department

Twenty-seven employees were able to continue in their jobs. Five were permitted to continue as bus operators when they were not taking insulin.

TABLE 2*

ABSENTEEISM IN A TRANSIT SYSTEM FEBRUARY TO OCTOBER 1951
(Number of days absent per employee per year)

	Sickness	Accident	Nonmedical
Total group	11 81	0 69	7.10
Diabetics	26 2	0 80	4 60

* Data from Brandaleone and Friedman, 1953 [1]

One patient who had pulmonary tuberculosis was unable to work because of it

In an effort to evaluate the efficiency of the diabetic in industry the three following factors were studied: (1) ability to work, (2) absenteeism and (3) caliber of work as defined by accident rate

(1) *Ability to work.* Twenty-seven employees were able to continue in their jobs Five were permitted to continue as bus operators when they were not taking insulin One
work because of it
limit Two resigned ... patients
were employed at the time of this study

(2) *Absenteeism* A meticulous study was made of absenteeism in a large transit system for the 9-month period from February to October 1951. Absenteeism was divided into 3 categories illness, accident, and nonmedical. Five of the 40 diabetics were not employed during this period of study, having been pensioned previously The remaining 35 were compared to the total group, and the results are shown in TABLE 2

The absence for sickness of the diabetic group requires a word of explanation Two individuals were absent for long periods of time. one patient with pulmonary tuberculosis was absent the entire 9-month period of study because of

The possibility of insulin reactions required the reassignment of 8 of these 10 workers to jobs where they were not likely to injure themselves or others. The other 2 diabetics who were doing shift work could not be adequately controlled because of the irregularities of their working hours

Much of the data presented by Wade demonstrated the importance of an adequate occupational health program[4] Early detection of asymptomatic cases was most frequently the result of periodic health examinations Proper education of the diabetic is of vital importance

Twenty nine and eight tenths per cent of all the diabetics had cardiovascular renal disease, while 31 1 per cent of them were suffering from obesity It was Wade's opinion that diabetics should not be hired where normal advancement requires rotation through assignments necessitating the operation of motor vehicles or fast-moving or heavy machinery, nor should shift work be essential in the course of their careers[4] Failure to assume a fair share of the "graveyard" shift is not looked upon favorably by fellow workers. The desire of most shift workers to rotate at relatively short intervals makes an adjustment of eating and insulin schedules complex and incompatible with optimal diabetic control

On the other hand, diabetes does not preclude productive employment, as evidenced by the experience reported in Wade's study

Improvement in the employment of the diabetic depends on the education of: (1) the diabetic himself, (2) the family physician, (3) industrial management, and (4) the industrial physician The use of oral preparations has removed most of the objections of industry However, an understanding of the problems of the diabetic will reduce further any existing objections to employing diabetics

The diabetic must understand his limitations in industry if he requires insulin or suffers with complications of his disease He must be taught that proper care of his disease, including close cooperation with his family and industrial physicians, is the best way to insure his ability to continue in his work

The American Diabetes Association states that the poorly controlled, uncooperative diabetic should be refused employment, while the well-controlled,

[illegible] performed by [illegible] In addition, the patient's physician should be in communication with the industrial physi-

[illegible]

cally by both the family and the industrial physician

The results of studies in industry show that the diabetic may be compared to the nondiabetic in his ability to work, the caliber of work, and his absentee record, except in a few isolated instances.

The industrial physician is an important factor in the care of the diabetic in industry This practitioner must be alert to the condition of the employee, the

pated in insurance and other employee benefits. The major conclusion drawn from these data was that "large companies have a more enlightened approach to the subject"

In a recent publication, Wade[3] reported a study of 266 diabetics in an employee population of 20,466 (an incidence of 1 3 per cent). This group of diabetics had an average age of 42.6 years. Seventy-five per cent of the cases were discovered after 40 years of age. Work assignments had usually become

TABLE 4*

RESPONSE TO A QUESTIONNAIRE SUBMITTED TO INDUSTRY

1 Number of employees		1,871,640	
2 Number of diabetics		2,658	
3 Do you do routine preplacement physical examinations?			
	Yes.	123	97
	No	3	2
	Did not answer:	1	1
4 Does this include a routine urine examination?	Yes·	106	83
	No·	21	17
5 Do you employ known diabetics?	Yes	88	69
	No	31	25
	Did not answer.	8	6
A If not, is it based on·			
(1) Previous poor experience?		17	13
(2) Insurance reasons?		16	13
(3) Statistics indicating unreliability?		4	3
(4) Other reasons?		5	4
Note 11 gave more than one answer		11	9
B If you do, what is your experience as to reliability and absenteeism			
(1) Satisfactory?		65	51
(2) Unsatisfactory?		7	6
(3) Same as average worker?		60	47
Note 44 gave more than one answer		44	35
(4) Do your diabetic employees participate in insurance and other employee benefits?	Yes	110	87
	No·	0	0
	Did not answer	17	13
(5) Do you make any concessions as to rotation of shifts?	Yes	35	28
	No	68	53
	Did not answer.	24	19

* Modified from· Committee on Employment of the American Diabetes Association[2]

stabilized and were not incompatible with the diabetic state. In a very few instances the advent of diabetes was the cause for job reassignment.

Wade commented that the workers continued satisfactorily in both sedentary jobs and heavy physical labor.[3] Approximately 10 per cent of the total group

Only 10 workers (3.2 per cent) were restricted because of their diabetes alone

The possibility of insulin reactions required the reassignment of 8 of these 10

Much of the data presented by Wade demonstrated the importance of an adequate occupational health program[8] Early detection of asymptomatic cases was most frequently the result of periodic health examinations. Proper education of the diabetic is of vital importance

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The American Diabetes Association states that the poorly controlled, uncooperative diabetic should be refused employment, while the well-controlled, cooperative diabetic is a good employment risk

The attending physician should understand the type of work performed by his patient so that he may be able to control the patient's disease In addition, the patient's physician should be in communication with the industrial physician, who better understands the problems on the job

The education of management in industry is most important The studies of the Committee of Employment of the American Diabetes Association have

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jol

of restrictions were imposed for cardiovascular-renal disease rather than for diabetes; these restrictions were the same in other workers with similar vascular disease without diabetes Restriction of work assignment was sometimes necessitated by concomitant vascular disease. Only 10 workers (3.2 per cent) were restricted because of their diabetes alone.

and should report periodically for physical examination and medical evaluation"

Summary

Education of the diabetic patient, of the family and industrial physician, and of industrial management will lead to a better understanding of the diabetic employee.

If the diabetic is well controlled and cooperative and presents himself for periodic examinations, he may be useful to his employer and to himself

References

1 Brandaleone, H & G J Friedman 1953 Diabetes in industry Diabetes 2(6) 448–453

2 Beardwood, J T et al 1957 Committee on Employment of the American Diabetes

3

4

Federal Service CSC Form 533 Washington, D C

accuracy of his control, and his ability to do his job; he must also be able to recommend proper job placement He must supervise the diabetic and be sure that the employee has periodic supervision by his own physician. Close supervision and cooperation will help prevent the complications of the disease or indicate early the presence of a complication for prompt therapy.

The industrial physician must always be alert to the development of diabetes in an employee and recommend the employee for prompt and proper therapy by his family physician.

The diabetic seeking employment should submit to a careful preplacement examination and, later, to regular periodic examinations to assure adequate diabetic control and the absence of complications. On the basis of preplacement examinations, proper job placement may be made.

On the basis of a more enlightened attitude, the objections to employing diabetics are minimized to negligible proportions.

A more liberal attitude in the employment of diabetics may be seen from a recent report of the United States Civil Service Commission, Washington, D C, which has published the pamphlet *Employment of Diabetics in Federal Service*,[4] which states

"The U. S Civil Service Commission believes that persons with controlled diabetes may be good employees and that it is good business to hire them. Regardless of this condition a diabetic is capable of safe and efficient service in an appropriate job, provided satisfactory control of his condition is maintained

"Mild diabetics who require no insulin for the control of their metabolic disturbance present no special difficulty in regard to placement They are ordinarily capable of performing any type of work for which they are otherwise qualified.

"More severe diabetics who require insulin and a regulated diet are acceptable for appointment to many positions, including arduous-duty positions provided medical evidence shows the condition is under control. When medical evidence is furnished to show the condition is properly controlled, the Commission will accept the individual's application for consideration for positions which do not require work at heights or around dangerous power-driven machinery, work involvin[illegible]
environmental situatio[illegible]
or others in the event [illegible]
Some individuals with less severe and properly controlled diabetes are given favorable consideration for motor vehicle operator positions, particularly those involving incidental driving

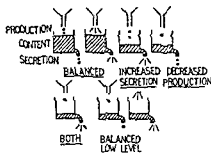
"More severe diabetics who require insulin and a regulated diet but who are uncooperative and/or poorly regulated are not recommended for Federal employment There is a small group of unstable diabetics who are cooperative and [illegible] [illegible] medical supervision, but in whom it is difficult to main-
tain
ment [illegible]
cation is made

"Diabetics should carry cards or tags at all times identifying their condition.

"Diabetics should be under the guidance of their clinic or personal physician

both insulin production and insulin secretion It is also well to appreciate that

concentration of insulin per islet cell can occur Hence, an additional factor may influence the insulin content of the pancreas and, conceivably, the granulation of islet cells This is the total volume of the islets If the total amount of islet tissue increases, then the insulin content of the pancreas increases, provided that the concentration per cell remains the same If the total insulin content of the pancreas remains the same, but the total volume of islet tissue is increased, then the concentration per cell will be reduced, and the granulation of the cell also will be reduced Changes in the amount of acinar tissue can



cretion are balanced

greatly influence the concentration of insulin in the pancreas as a whole, but

directly, as can the secretion of the exocrin glands However, some information about the secretion of insulin can be obtained indirectly by noting histological changes in the islet cells or by measuring changes in the insulin content of the pancreas or in the insulin that has entered the blood

If the production of insulin by the pancreas does not change, then estimating the level of pancreatic insulin provides a measure of secretion. Secretion may be estimated also by measuring the changes in the level of insulin in the blood This blood level of insulin depends on the balance between the secretion and removal of insulin, as shown in FIGURE 2. The reservations are similar to those

Part II. Recent Advances in Studies of Insulin Secretion and Cert
Metabolic Effects

ISLET CELL FUNCTION

R E Haist

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The islets of Langerhans are small masses of specialized cells lying in the e ternal secreting tissue of the pancreas In the rat, they make up about 1 p cent of the total pancreas and, if all the insulin is assumed to be in the islet then the insulin content per gram of islet tissue is about 300 U /gm., that i somewhat more than 1 per cent of the islet is insulin

The islets make and secrete insulin The evidence for th

pancreas and the granulation of the beta cells when they are stained with aldehyde-fuchsin stain I shall limit this discussion to a con sideration of the function of beta cells

The changes in the histological appearance of the beta islet cells may be gross morphologic alterations, as one might observe with hydropic degeneration or extensive atrophy or disappearance of beta cells or changes in the degree of granulation of their cytoplasm when stained in a particular way In addition, changes concerned with the Golgi apparatus may help in an assessment of islet cell activity The strictly histological indices are considered by others else- where in this monograph

In some species there is good correlation between the degree of granulation of the beta cells and the insulin content of the pancreas *Granulation studies* therefore give somewhat the same type of information as studies of the insulin content of the pancreas Because of this, we shall consider these two types of investigations together The insulin content of the pancreas and the state of granulation of the islet cells, by themselves, tell little regarding islet activity but, taken in conjunction with other measurements or taken under different experimental conditions, this content may give a great deal of valuable in- formation concerning islet function A reduction in the insulin content of pancreas or in the granulation of islet cells may result from some disproportion between the production and secretion of insulin (FIGURE 1) This effect may be due to an increased secretion of insulin that is out of proportion to its produc- tion, or to a decreased production of insulin that is out of proportion to its secre- tion The disproportion accounts for the change, but it is conceivable that a level may be maintained despite changes in secretion if secretion and production remain balanced Thus, sudden changes in secretion or excessive changes would be more likely to cause changes in the insulin content of pancreas or in the granulation of islet cells than slow changes With slower chang it seems poss neitl rdingly.

Ir

duce a fall in blood sugar level, this might be because it failed to stimulate insulin secretion, or because the secretion of something else (for example, gluca-

late the islets Also, it is difficult to make these observations quantitative in relation to insulin secretion

Insulin injections will cause the glucose-6-phosphatase activity of the liver to be reduced Changes in glucose-6-phosphatase can be used as corroborative evidence of insulin secretion if the experiment is of sufficiently long duration However, these changes can help to confirm only what is suspected from other information

Direct visualization of the islets in living animals has not been used extensively in studies on islet secretion Many years ago O'Leary[2] described changes

alterations in the total islet mass when the animal as a whole has been treated,

These various procedures have been used in studying the secretory function of the islets of Langerhans When properly used, measurements of the insulin

observations on the blood insulin level, then the interpretation would be aided Using the various procedures, it has been possible to gain some idea of factors influencing the activity of the islets While evidence of the importance of certain regulating factors is good, yet the way in which they bring about secretion is not clear, nor is the final common factor, if there is one, known with certainty (FIGURE 3) One may say, however, that the fundamental final common factor in the regulation of insulin secretion is probably humoral The evidence indicates that nervous regulation is not of fundamental importance, since the pancreas can regulate insulin secretion remarkably well even though denervated or transplanted One humoral factor of importance must be glucose It is conceivable that the level of glucose in the blood going to the pancreas is the fundamental stimulating factor insofar as immediate secretion is concerned Another factor of importance must be the level of insulin in the blood Insulin administration depresses islet function, but it is possible that the insulin effect is primarily on insulin production rather than secretion, since the insulin content of pancreas falls, presumably because, initially, the production of insulin is reduced to a greater extent than its secretion The pituitary gland does not seem to be fundamental to the regulation of insulin production and secretion, since there is evidence that large changes in islet activity can occur in hypophysectomized animals. However, it is conceivable that the basic stimulating factor is glucose, and that other agents such as insulin, anterior pituitary sub-

mentioned for the insulin content of the pancreas. However, at present it is easier to investigate the relationship between the production and secretion of insulin in the pancreas than between the secretion and removal of insulin from blood. This removal must also involve renal excretion, which further complicates the picture. The insulin content of peripheral blood by itself, therefore, does not provide a very satisfactory index of insulin secretion.

A better indication of insulin secretion would be the total insulin content of the blood leaving the pancreas. The islets constitute about 1 per cent of the pancreas, and the circulation to the islets is not separate. Since one cannot separate the blood flow to the islets from that to the acinar tissue and, also, since fluid may be removed from blood as it passes through the pancreas, it is necessary to know the amount of blood leaving the pancreas and the insulin contained therein. If it were possible to measure the total amount of insulin leaving the pancreas, then changes in this value would satisfactorily indicate changes in insulin secretion, provided there were no differences in the amounts of insulin held by the other tissues of the pancreas. However, any procedure permitting such measurements must be done acutely in anesthetized animals. The op-

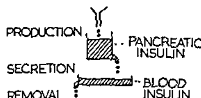


FIGURE 2. Diagram to show that the insulin level of the blood depends on the relation between the secretion of insulin into the blood and its removal (as by utilization, destruction, or excretion). The same reservations apply as for the insulin content of pancreas.

erative procedures and the anesthetic must produce changes in blood flow and changes in function that are not easy to compute. Not the least of the difficulties in this and in the previous method, as well as in the one to follow, is the accurate quantitative measurement of the insulin content of the blood. The difficulties in estimating this are well known and will not be discussed here.

Studies on the isolated perfused pancreas also might permit a calculation of the total amount of insulin leaving the pancreas and hence of islet secretion, but in these experiments the conditions are even more abnormal. One could expect that factors involved in the production of insulin might be interfered with and that, in addition, the circulation to the islets would probably be affected in a way difficult to predict.

Other studies not directly involving insulin measurements may provide information relating to the secretion of insulin. These investigations involve the measurement of changes in certain materials or activities and the comparison of these with the changes produced by insulin. For example, when sugar is infused directly into the pancreatic artery in small amounts the general blood sugar level will fall, whereas this amount of sugar injected elsewhere may

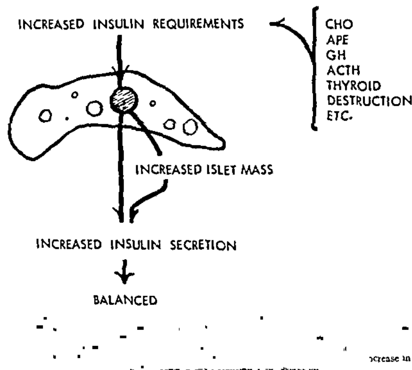
This fact would

by the pancreas

ry failed to pro-

fortunately, very little is yet known concerning the way this circulation is regulated and what relation it bears to the activity of the islets.

The cells of the islets seem to be able to vary the secretion of insulin within limits according to the need for it (FIGURE 5) Ordinarily, then, there may be a large variation in the secretion of insulin by the existing islet mass. In addi-



crease in

tion, the mass of islet tissue can be increased above the pre-existing levels In

tion while maintaining a reserve of function

It will not be possible to deal with all aspects of the regulation of insulin secretion at this time, but an attempt will be made to review briefly the evidence for some of the regulating factors The factors influencing the islets may be divided in a general way into those that reduce islet activity and inhibit islet growth and those that increase islet activity and enhance islet growth. The term growth is used here, in an arbitrary way, simply to mean an increase in the

stances, cortisone, and thyroxine, produce their effects by altering the ease of access of glucose to the cell or by influencing the sensitivity of the insulin-secreting mechanism to glucose. Whatever the fundamental final common path may be, many factors do influence islet activity and growth, and some of these still act in the absence of the pituitary gland. One further factor must

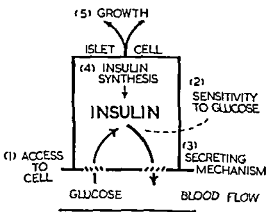




FIGURE 4 India ink injected vessels of an islet, showing the rich blood supply

considered to be due to reduced activity of the islets, a conclusion substantiated by the fact that when the insulin administration was stopped the animals temporarily became diabetic

Restriction of the food intake also reduces the activity of the islets and islet growth When rats were fasted for 1 week the insulin content of the pancreas was reduced to about one-half the normal value (FIGURE 8),[4] when insulin was injected into the fasting rats, the insulin content of pancreas was reduced to much lower values[5] This observation would seem to indicate that the effects

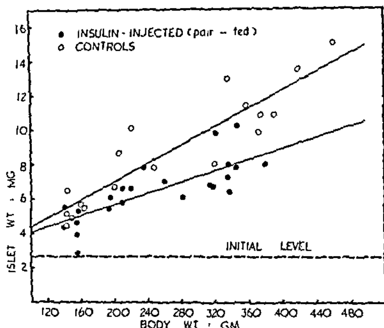


FIGURE 7 The effect of insulin administration on the growth of the islets of Langerhans Reproduced with permission from the *American Journal of Physiology*[4]

of fasting and of insulin administration are in the same direction and would

a reduction in the concentration of insulin in the islet cells However, when young rats were fed amounts of a diet just sufficient to maintain their body weight but insufficient to permit body growth, the islets also failed to grow (FIGURE 9)[6] It is concluded, then, that restriction of caloric intake reduces the insulin content of pancreas, inhibits islet growth, and reduces the activity of the islets, a conclusion that is supported by reports in the literature of starva-

total amount of islet tissue without specifying whether this is a consequence of an increase in the number of islet cells or of an increase in the size of individual
involved in the increase.
can be reduced, and/or
uate amounts of insulin,
by the restriction of caloric intake, by the restriction of carbohydrate intake and by removal of the pituitary gland, and that islet activity can be increased and islet growth enhanced by increasing the carbohydrate or carbohydrate-forming substances in the diet, by infusing sugar intravenously, by administering anterior pituitary extracts, growth hormone, or ACTH, by administering thyroid hormone, by injecting steroids of the adrenal glands or of the sex glands, or by administering certain sulfonylurea compounds.

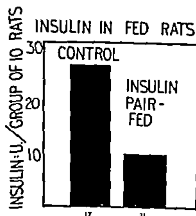
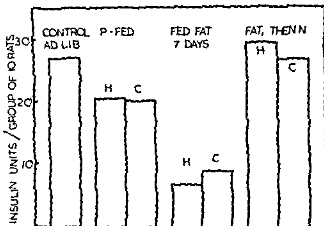


FIGURE 6 The effect on the insulin content of pancreas of adequate insulin administration in fed rats The insulin-injected rats were pair-fed with the controls [28]

Investigations concerning a number of different endocrine glands indicate that the injection into an animal of the hormone produced by a particular gland reduces the production of that hormone by the gland When adequate amounts of insulin are given over a period of time to rats, several effects are noted
First,
islets.
strate
in the rat is associated with a reduction in the insulin content of pancreas The injection of 1 to 3 U protamine zinc insulin daily for 1 week caused a marked reduction in the insulin content of the pancreas (FIGURE 6) This reduction in insulin content cannot be ascribed to a corresponding reduction in islet mass but rather to a reduced concentration of insulin per unit of islet tissue. In growing rats it was not possible to demonstrate a reduction in the total amount of the islet tissue below the initial levels by the administration of insulin, but the normal increase in islet tissue was inhibited (FIGURE 7) [3] Insulin thus reduced the specific granulation of beta islet cells, reduced the concentration of insulin in islet cells, and inhibited the growth of the islets These effects are

The effect of dietary constituents on the volume of islet tissue was demon-

data). In 3 to 5 weeks the animals on the carbohydrate supplement and on the protein supplement showed a significant increase in islet volume, whereas *those on the fat supplement did not. From these experiments it would appear* that there must be sufficient amounts of carbohydrate or carbohydrate-forming substances in the diet if insulin production is to be maintained and islet growth is to continue



It is known from other work that the anterior pituitary gland exercises an important influence on carbohydrate metabolism and on many endocrine glands Removal of the pituitary gland stops the growth of the rat, and the amount of food the animal eats is lessened After a period of time following removal of the pituitary, the insulin content of the pancreas is diminished when compared to the values obtained in those rats receiving as much food as they *would eat, but it is not reduced in relation to the levels in control animals re-*ceiving the same caloric intake (FIGURE 10) *Moreover*, if the hypophysectomized rats are fed a diet very rich in fat, the insulin content of the pancreas is reduced to much the same degree as in control animals, and the refeeding of a balanced diet following a period of 1 week on the fat diet brings back the insulin content of the pancreas to normal levels or even above them[7] It would appear from this that, in growing rats, the removal of the pituitary gland does not *prevent the changes in insulin production caused by feeding fat*, that is, by

tion diabetes or "hunger" diabetes, a diabetes of transient type following a period of starvation or undernutrition

There is a good deal of evidence to indicate that restriction of carbohydrate or carbohydrate-forming substances will produce an effect similar to that of

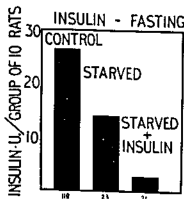


FIGURE 8 The effect of fasting and of insulin plus fasting on the insulin content of pancreas [28]

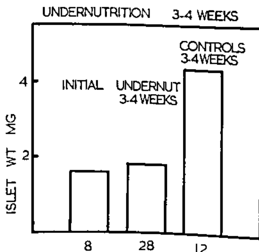


FIGURE 9 The effect on islet weight of keeping rats on a diet just sufficient to maintain their body weights (undernutrition) Control rats were fed ad libitum

reduced caloric intake When rats are fed a diet very high in fat there is a reduction in the insulin content of the pancreas that is as great as in complete starvation [4] Here, too, the effect is enhanced by the simultaneous administration of insulin [6] In experiments in which equicaloric amounts of fat and carbohydrate were fed, the animals fed fat showed a much lower insulin content of pancreas than those fed carbohydrate

Cortisone, one of the products of the adrenal gland, the target organ for ACTH, also caused a significant increase in islet volume. The effect of thyrotropic hormone was not tried, but the administration of desiccated thyroid gland for

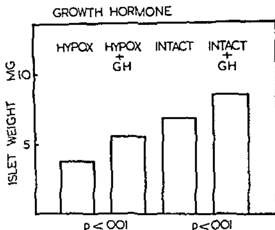


FIGURE 12. The effect on islet weights of growth hormone administration. HYPOX = hypophysectomized rats, GH = growth hormone injected rats, INTACT = rats with pituitary glands intact.

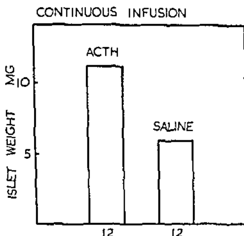


FIGURE 13. The effect of the continuous intravenous infusion of ACTH on islet weight in rats. ACTH = ACTH-infused rats, SALINE = saline infused control rats.

periods longer than 40 days led to significant increases in islet tissue.[12] One report indicated that the insulin content of the pancreas was increased.[13] The effect of the gonadotropic hormones was not tried, but the hormones of the target organs in the female (that is, the female sex hormones) and similar steroids did give significant increases in islet tissue. This held for the estro-

restricting the carbohydrate intake, hence, the islets can alter their activity in relation to the need for insulin in the absence of the pituitary gland. In addition to this, it is found that removal of the pituitary gland does not cause a significant atrophy of the islets in young rats (FIGURE 11), although it does prevent further islet growth under ordinary circumstances

It is possible to have a great reduction in the insulin content of pancreas without any marked decrease in the amount of islet tissue but, when one considers those factors that increase the insulin content of pancreas considerably above the normal values, it is found that the conditions increasing the insulin content of the pancreas in the rat also lead to an increase in the total amount of islet tissue Whether or not this holds true for endocrine glands in general, it seems to be true, insofar as the endocrine pancreas is concerned, that whenever there is a sustained increase in the concentration of hormone in the gland

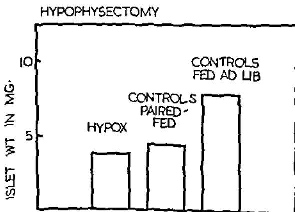


FIGURE 11 The effect of hypophysectomy (28 to 130 days) on islet weights of rats The rats of one control group were paired fed with the hypophysectomized rats and the rats of the other control group were fed ad libitum

an increase in the tissue producing the hormone has been found A decrease in the hormone level in the gland, however, need not be associated with any measurable change in the total amount of hormone-producing tissue

Daily injections of saline extracts of the anterior pituitary gland into rats have been reported to cause an increase in the insulin content of the pancreas[8] and an increase in the total amount of islet tissue[9] The latter finding has been substantiated, and it has been shown in addition that certain highly purified anterior pituitary principles (growth hormone, adrenocorticotropic hormone (ACTH), and prolactin) have this effect also Significant increases in islet tissue were found after the administration of each of these materials for periods of several weeks. The effect of growth hormone was obtained using subcutaneous injections for 4 weeks, but the best effect of ACTH was obtained when the material was given by continuous intravenous infusion for 1 week FIGURE 12 sh[illegible]
and i[illegible]
week,

When one follows the blood sugar level in rats injected continuously with glucose for a period of 7 days, it is found that the blood glucose level goes up initially but that, within about 24 hours, the level returns to the normal range unless the condition of the animals declines. (B Kinash and R. E Haist, unpublished data) This can be seen in FIGURE 15. Despite the continuation of the infusion, the blood sugar level is not elevated for long. Nevertheless,

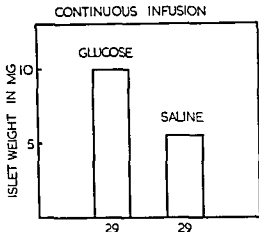


FIGURE 14 The effect of the continuous intravenous infusion of glucose on islet weight in rats GLUCOSE = glucose infused rats, SALINE = saline-infused rats.

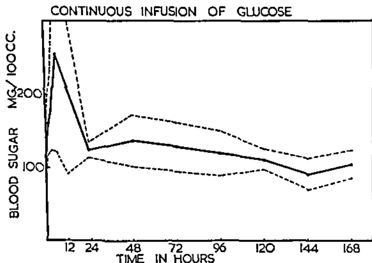


FIGURE 15 Blood sugar levels in rats continuously infused with glucose (0 8 cc /hour of 40 per cent glucose in 0 5 per cent saline). Mean values and standard deviations are shown

gens, estradiol benzoate [illegible] but administration of testoste[illegible], but increase in islet tissue[14] [illegible]ce any [illegible] the report that the administration of certain estrogens increased the insulin content of the pancreas[15] It seems evident from what has been outlined that the administration of a number of pituitary trophic principles or the hormones of their target organs leads to an increase in the total amount of islet tissue. *There is always* the possibility that this effect is mediated by one particular hormone, namely, a pancreatrophic pituitary hormone However, it can be shown that several of these hormones or principles can produce their effects on the islets in the absence of the pituitary gland *Indeed*, it is often possible to obtain a more pronounced effect in the hypophysectomized animal. Very good and significant increases in the total amount of islet tissue, in the absence of the pituitary gland, were obtained with growth hormone, ACTH, thyroid, and cortisone The effect of the estrogens was not tried in the hypophysectomized animal

It would appear, then, that a number of different, highly purified substances can stimulate the growth of the islets and *increase* the amount of islet tissue above the normal level It is also evident that the stimulating effects of a number of these substances can be obtained in the absence of the pituitary gland, hence, it is unlikely that their *effects* are med[illegible] [illegible] the amount of islet tissue are in some way consequences of sustained increased insulin requirements and hence of increased islet activity Studies of the insulin activity of blood[16-18] indicate that growth hormone administration for several days increased the insulin activity of blood in some species, but not in others Investigations with pancreatic transplants in the dog[19] also gave evidence for increased insulin secretion following the administration of growth hormone.

One important effect on the islets is exerted by *carbohydrate* or carbohydrate-forming substances Carbohydrate in the diet is essential for the maintenance of the insulin content of the pancreas and the growth of the islets In addition, it can be demonstrated that, when large amounts of glucose are given by continuous intravenous infusion, the islet volume *quickly* and dramatically increases (FIGURE 14)[20] At the same time, there is some uneven degranulation of the beta cells of the islets during the course of the injections, but insulin estimations have not been done This effect of a large *glucose* load on the islets is interesting because there is a good deal of evidence in the literature to indicate that the islets may be stimulated directly by increasing the glucose level in the blood going to the pancreas. In the isolated *perfused* pancreas it was found that increasing the glucose level of the perfusate led to the appearance of detectable amounts of insulin, indicating insulin secretion.[21] It was reported also that the cat given glucose intraperitoneally over a *period of* time exhibited degenerative islet changes and became permanently diabetic,[22] and it has been demonstrated that glucose given into the pancreatic artery led to a *fall* in blood sugar level and, after a period of time, to hyperplastic islet changes[23] It has been indicated also that after glucose administration there may be as much as a tenfold increase in the plasma insulin level[24]

While under most circumstances those factors stimulating islet activity and growth also raise the level of blood sugar, in one experiment the islets appeared to be stimulated and to grow, even though the blood sugar was apparently not elevated and the insulin requirements apparently were not increased (FIGURE 18). This was when the sulfonylurea compound, BZ-55 (carbutamide) was given daily to rats for a period of 3 to 5 weeks.[24] The total amount of islet tissue in the pancreas was increased under these circumstances, and other evidence supports the conclusion that insulin secretion from the pancreas was stimulated by the administration of the drug. The blood sugar level of animals similarly treated was reduced, and the clinical evidence would lead one to conclude that the requirements for insulin would be reduced also. Hence, we can-

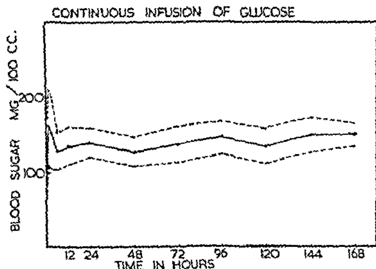


FIGURE 17. Blood sugar levels in hypophysectomized rats continuously infused with glucose. Mean values and standard deviations are shown.

not ascribe the stimulation of the islets or the increase in islet tissue obtained under these circumstances to an increase in blood sugar level or sugar load or to an increase in the requirements for insulin. This is possibly an instance of direct stimulation of islet activity by a drug, but we cannot be dogmatic about how the stimulating effect on the islets is obtained. In the rat the granulation of the beta islet cells is reduced as a result of the administration of carbutamide and, in the dog, the insulin content of the pancreas is greatly diminished. The insulin content of pancreas subsequently returns to normal levels despite the continuation of the treatment. This might result from an increase in the total amount of insulin-secreting tissue or merely from an increase in insulin synthesis in the existing islet cells that is sufficient to balance secretion.

It has been seen that certain factors reduce islet activity and islet growth. These are the administration of adequate amounts of insulin, restriction of food

... glucose load, or, possibly, access of glucose to the inte... of the islet cell, or sensitivity of the insulin-secreting mechanism to gluc... Since this effect of glucose is such a good one, and since there ...

... of glucose in the absence of the pituitary gland When glucose was co... tinuously infused into the hypophysectomized rat, it was found (Kinash a... Haist, above) that a significant increase in islet tissue was not obtained, ev... though the amounts of glucose given were similar to those injected into ... intact rats (FIGURE 16)

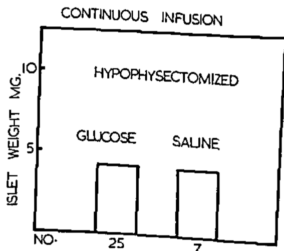


FIGURE 16 The effect on islet weight of the continuous intravenous infusion of glucose and saline into hypophysectomized rats

The studies on the insulin content of pancreas would indicate that in the hypophysectomized rat there can still be regulation of the activity of existing islet cells in relation to changing insulin requirements. The basic regulation is still present. However, in the absence of the pituitary gland, islet growth is not stimulated by the glucose load. The blood sugar level does not rise as high initially in the hypophysectomized animals infused with glucose, but as shown in FIGURE 17, the sustained values are as high or a little higher than in the intact rats infused in the same way (Kinash and Haist, above) The loads and the levels of glucose were comparable, and yet the increase in islet tissue was not obtained when the pituitary gland was removed

... lating effects of ... pancreatic artery could still be obtained in the absence of the pituitary gland

increase in islet tissue In the normal individual an increase in insulin requirements will be balanced by an increase in insulin secretion and an increase in islet tissue. In the diabetic, this compensatory increase in insulin secretion and islet tissue, if it occurs, is not sufficient to balance the insulin requirements. Consequently, one way of thinking of diabetes is to consider it as, basically, a disorder in homeostasis or a disorder in compensation

Not all species respond in the same effective manner as the rat to greatly increased insulin requirements. Most of the effects considered were obtained in growing rats, and one would expect that similar effects would be obtained in other species This is true, but not necessarily to the same degree Young

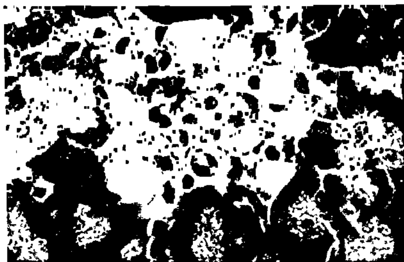


FIGURE 19 Photomicrograph of an islet from a dog that received 11 daily injections of an extract of the anterior pituitary gland Reproduced with permission from the *American Journal of Pathology* [26]

growing dogs and cats probably are much like the rat, but in certain adult dogs and cats those factors that were shown to stimulate islet secretion and growth in the rat caused overstimulation, exhaustion, and degeneration of the islet cells This can be illustrated by showing the effects of injections of anterior pituitary extracts In the rat, these caused the islets to grow With daily injections of anterior pituitary extracts in the dog, proliferation of islet cells was evident in some instances, hence growth was occurring, but was not sufficiently rapid and extensive to prevent the development of diabetes, and degeneration of the islets occurred (FIGURE 19) [26] If insulin was given along with the injections of anterior pituitary extracts, then the diabetes was prevented, as was the degeneration of the islets (FIGURE 20) [27] Similarly, the excessive stimulation and degeneration of the islets could be prevented by fat feeding or fasting during the period of APE injections [28] The reason for mentioning these effects in

intake, restriction of carbohydrate intake, and removal of the pituitary gland. Other factors increase islet activity and enhance islet growth. These are administration of pituitary substances, or administration of hormones of the thyroid adrenal, or female sex glands

an

tion [illegible]

according to the requirement for insulin whether the amount of islet tissue be large or small. When hyperinsulinism occurs, therefore, it is an evidence of

FIGURE 18 The effect of the administration of BZ-55 for 3 to 5 weeks on the islet weights and pancreas weights in rats C = mean values for control rats BZ-55 = mean values for BZ 55-treated rats Reproduced with permission from the *Canadian Medical Association Journal* [26]

some abnormality in the mechanisms regulating insulin secretion rather than in the amount of islet tissue

There is evidence that, under normal circumstances, insulin requirements are

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nevertheless good If this increase continues, an increase in the total amount

of insulin-secre

then, when the

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requirement, as with excessive insulin administration, the growth of the islets is diminished. Thus, in growing rats at least, the islets of Langerhans are responsive structures important in homeostasis It is felt that an important part of the response to a more sustained large increase in insulin requirements is an

life, there is still a considerable amount of apparently normal islet tissue, and the insulin content of the pancreas may be within the normal range. Since the individual is diabetic, there must be a relative lack of insulin. If the insulin lack in these diabetics were due to an increased utilization or destruction of insulin, that is, increased insulin removal, then this would greatly increase the insulin requirement. Since the requirements are not being met, there should be evidence of greatly increased sustained activity of the islets, namely, signs of either islet cell degeneration or islet growth. In some of these adult human diabetics there is little or no evidence of increased stimulation of the islets. If the demands for insulin in these patients are indeed excessive, nevertheless they are not met by normal compensatory responses. This would suggest that there is not a greatly increased requirement for insulin but, rather, that there is some interference with the secretion of insulin or the stimulation of this secretion.

However, islet atrophy is not extensive, and the insulin content of the pancreas is not greatly reduced in the pancreas of these patients. This would suggest further that the islets are still being stimulated, although this stimulation appears to be less than it should be at normal blood sugar levels.

The tentative conclusion is that in certain adult human diabetics at normal blood sugar levels the stimulation of insulin secretion by the islets is subnormal. Whether this is due to altered access of glucose to the islet cell or to altered sensitivity of the insulin-secreting mechanism to glucose or to some change in the secreting mechanism itself, or to an altered circulation to the islets is not known. It seems unlikely that it is due basically to a primary reduction in insulin synthesis, otherwise the insulin content of the pancreas should be low. While there is failure of islet growth to compensate for the insulin need, this would be a secondary effect consequent on the failure of stimulation of the islet cells.

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... tissue and, hence, insulin secretion. In the adult dog, som growth of the islets does occur, but this is insufficient, the islet growth is no sufficiently rapid, so that the increased insulin requirements put excessive de mands on the islet tissue and the cells degenerate In the adult dog, then the compensatory islet increase is not adequate, and diabetes results.



FIGURE 20 Photomicrograph of an islet from a dog receiving insulin in addition to in jections of an extract of the anterior pituitary gland for 11 days Reproduced with per mission from the *Journal of Physiology*[37]

Diabetes resulting from injections of anterior ...

... consequence of a continued large increase in insulin requirements in experimental animals is either an increase in the amount of islet tissue or degenerative changes in ...

... destruction of the beta cells, a very low insulin content of the pancreas and, one presumes, an absolute lack of insulin. In certain diabetics whose diabetes has its onset somewhat later in

ELECTRON MICROSCOPY OF THE ISLETS OF LANGERHANS*

Paul E Lacy and W. Stanley Hartroft
Department of Pathology, Washington University School of Medicine, St Louis, Mo

Knowledge of the synthesis, storage, and liberation of insulin by beta cells

bioassays indicate that amounts of extractable insulin in the pancreas are adequate, and histological studies reveal that numbers of beta granules are

that at least one important action of these new drugs is to release some of the same insulin from beta cells that was not released by the stimulus of high levels of blood sugar If this is true, then these drugs may be considered as keys that unlock stores of insulin previously inaccessible to the patient through the usual physiological mechanisms

The problem of why the drugs can act when hyperglycemia cannot seems to be clearly formulated, and answers may finally rest with the biochemists and enzymologists rather than with the pathologists However, if structural alterations in beta cells and islets share in the pathogenesis of diabetes in adults, morphologic studies, particularly at ultrastructural levels, may indicate pathways for the pursuance of solutions We do not have answers yet, but the questions have stimulated my colleagues and me to attempt the elucidation of finer structures of beta cells with the electron microscope and to follow their changes under various physiological and pathological conditions Abnormal-

in man

Alterations in Islets of Adult Diabetics

normalities Furthermore, those lesions that are demonstrable in the remainder of cases are not in any sense pathognomonic of the diabetic state because all may be found to certain degrees in the islets of some nondiabetics Even when these changes are present, we cannot be sure always that they are

he work reported in this paper was supported in part by Grant A-1226 from the Na-
Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md

20 KINASH, B & R E HAIST 1954 Can J Biochem. Physiol. **32:** 428

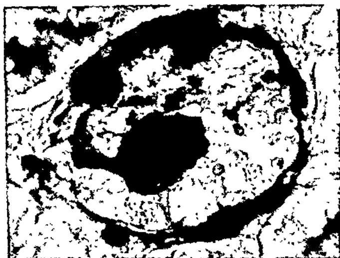


FIGURE 2 Hyalin appears black in this paraffin section of an islet from an adult diabetic (in frozen sections, lipid could also be demonstrated, as in FIGURE 1) Most of the hyalin is deposited in or around the capillary walls and appears to have narrowed the one in the center of the field almost to the point of occlusion Some hyalin droplets can be seen intracellularly Connective tissue stain ×800

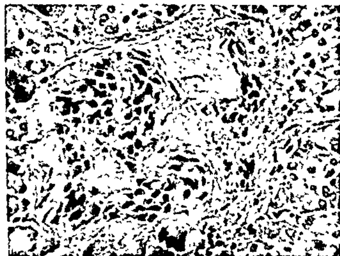
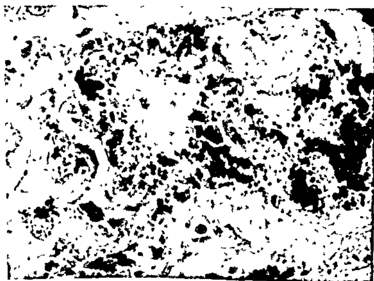


FIGURE 3 A mixture of hyalin and collagen is deposited in and around the sinusoids and beta cells of this islet from an adult diabetic The collagen appears to be fibrillar, the hyalin smooth The beta cells between have rather shrunken dark nuclei that may suggest some pyknotic change Hematoxylin-eosin stain, paraffin section ×800

Degranulation with hydropic or glycogenic vacuolation (or both) is found at autopsy in only 5 to 15 per cent of adult diabetics. These cases, in which amounts of extractable insulin are significantly low, represent but a minority of the total, in contrast to a majority of juvenile diabetics, in whom islets are reduced in number or sometimes even completely absent,[2] and in whom the insulin content of the pancreas is low However, in the remaining 60 to 75

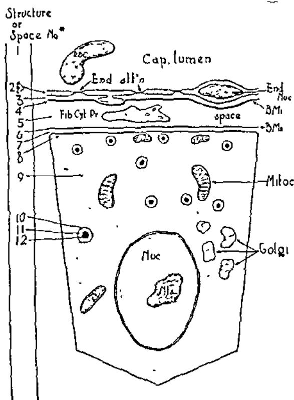
or collagen), and not infrequently all three of them, can be present within a



single islet Fat, in abnormal stainable form, and sometimes in large amounts, is probably the most ubiquitous Lipid deposits almost always accompany hyalin, to such an extent that we prefer to refer to this combination as one complex lipohyalin Lipid may often be overlooked or forgotten because the usual methods of examination, such as paraffin sectioning, dissolve it from tissues

Beta cells enmeshed within lipohyalin or collagenous bands along sinusoidal channels may appear to be relatively normal (FIGURE 4) They may be smaller than usual and their cytoplasm reduced Sometimes their nuclei are abnormally small and darkly staining (FIGURE 3), but secretory granules may be as numerous as in beta cells of nondiabetics similarly examined The temptation is strong to assume that abnormal deposits along or near basement membranes may act as barriers either to passage of glucose from blood to beta granule

a second (BM_2) by a wide space (5) in which processes of fibroblasts (Fib Cyt. Pr.) are encountered The second basement membrane (6) is closely related to islet parenchymal cells, from which it is separated by only a narrow space (7).



Under certain circumstances described below, this space may become wider than it is normally. Neither basement membrane dips between adjacent beta cells, although the second one (BM_2) appears to be more closely related to islet cells than to capillary endothelium. To enter a beta cell, its plasmal membrane (8) must be penetrated to reach the cytoplasmic ground substance

(to stimulate insulin release) or, conversely, to passage of insulin from beta cell to blood If either hypothesis has any basis, how do oral hypoglycemic agents overcome these hindrances,
by facilitating passage of ins
hyalin membranes of islets?
tion of various membranes and spaces, both intracellular and extracellular, through which substances from the blood stream must pass to reach beta granules and through which the latter must pass to reach blood



FIGURE 4 This nest of beta cells is surrounded by a meshwork of collagen and hyalin The nuclei stain somewhat darker than normal, but the cytoplasm is not reduced, and granules can be seen Gomori's chromium hematoxylin phloxin stain ×1000

Structures Separating Blood from Beta Granules

Electron microscopic studies of islets from a number of
us to represent
tween blood and
particular species, a general concept based on all will be presented. The figures and abbreviations in brackets in the next paragraph refer to the diagram in FIGURE 5.

Leaving the capillary lumen (1), the first barrier encountered is the endothelial wall of the vessel, which consists of an inner plasmal membrane (2a), a thin component of ground substance (2b) in which may be found scattered small mitochondria, and the outer plasmal membrane (2c). At frequently
endothelial cells are in almost
of capillary walls (End att'n.)
(3) from the first of two basement membranes (4, BM₁), which appears more closely related to the blood vessel than to islet cells This basement membrane (BM_1) is separated from

before leaving it but, after pathological stimulation in some species, empty granule saccules were found deep within beta cells. The latter observation suggests that insulin could leave its saccule at any site within the cytoplasm and traverse the ground substance toward the capillary in some soluble, non-

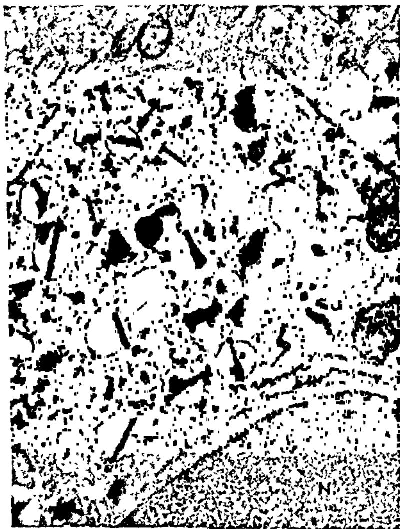


FIGURE 7 Electron micrograph of a normal beta cell from a dog prior to the injection of Orinase Many of the granules (*arrows*) have rectangular profiles and are enclosed in membranous sacs Ergastoplasm (ER) and mitochondria (M) are scattered diffusely throughout the cytoplasm N = nucleus ×24,000

(9) with its contained mitochondria (Mitoc) and Golgi vacuoles (Golgi). Insulin in beta granules (12) is reached by traversing first the thin but definite wall (10) of the small saccule in which the granule lies and next by crossing the space (11) that separates the granule (12) from its saccule wall. At least 5 membranes and 5 spaces separate granules from blood In theory, pathological alterations at any one or more of these 10 sites could interfere with insulin liberation

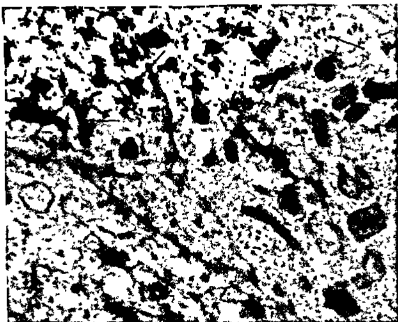


capillary ... and the granules

Although evidence that insulin is stored in beta granules is strong,[1] some also is probably

observed in

tions, it is q



a diabetic state that is coexistent with normal amounts of extractable pancreatic insulin and normal complements of beta granules with or without fatty, hyaline, or fibrotic change as encountered in adult diabetic man. Without this experimental model, which is so sorely needed, we must depend upon biopsy material obtained from man.

Variations in Form and Number of Beta Granules with Species and under Abnormal Conditions

In the foregoing, two pathological changes in islets have been considered as possible factors in the pathogenesis of diabetes in man, (1) reduction in insular

granular form. Granules have not been observed free of their enveloping saccules

By whatever mechanisms insulin reaches sinusoidal borders of beta cells, the membranes here in most species are irregularly thrown into a few, fine projecting processes or microvilli Granules have not been seen within villi, which suggests that at least somewhere in this vicinity, insulin must exist in nongranular solution These villi have been observed to increase in number following the application of certain stimuli,[2] as in glucagon administration in the

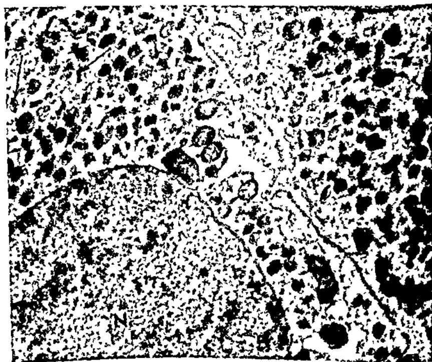


FIGURE 8. Electron micrograph of portions of normal beta cells of the cat The granules have a central dense structure embedded in a less dense homogeneous substance. The dense structure has a rhomboidal shape in some granules and a circular or elliptical shape in others N = nucleus ×24,000

rat (FIGURE 6). Perhaps under some conditions numbers and size of villi may be an index of rate of liberation of insulin from beta cells Villi could result from the fu

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of a small concavity Such a process could be termed reverse pinocytosis or, to int duce a single word for such an event emiocytosis (ejection from a cell).

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within the granule complex for each animal, is distinctly and characteristically species-specific. Studies are in progress in this laboratory to discover the ultrastructure of beta granules in normal man and to investigate the possibility of abnormal forms existing in beta cells of diabetics

Changes in granule content and the abnormal accumulation of glycogen that develop in beta cells of cats subjected to hyperglycemia following repeated intraperitoneal infusions of glucose solutions have been studied by electron

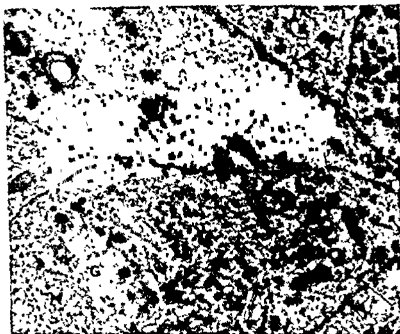
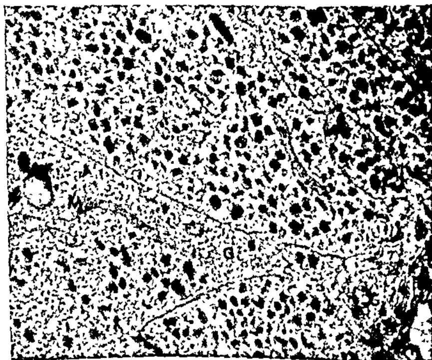


FIGURE 11 Focal accumulations of glycogen (G) are illustrated in portions of beta cells of a cat following repeated intraperitoneal injections of glucose The branching strands of glycogen are separated by clear spaces of fluid The beta granules (arrows) are decreased in number The ergastoplasm (ER) is abundant in some cells and has a lamellar structure N = nucleus ×18,000

microscopy Poles of cells adjacent to capillaries contained many granules, whereas opposite ones were filled with a lamellar type of ergastoplasm Abnormal deposits of glycogen were demonstrable by light microscopy in islets of injected animals Two patterns of deposition were evident by electron microscopy, in one, glycogen formed a meshwork of gray, branching chains scattered diffusely throughout the cytoplasm (FIGURE 10) Beta granules, although present, were fewer than in the controls The second pattern consisted of focal accumulations of glycogen that displaced surrounding organelles (FIGURE 11) Abnormal collections of fluid were apparently associated with some of these deposits, because the delicately branching strands of glycogen

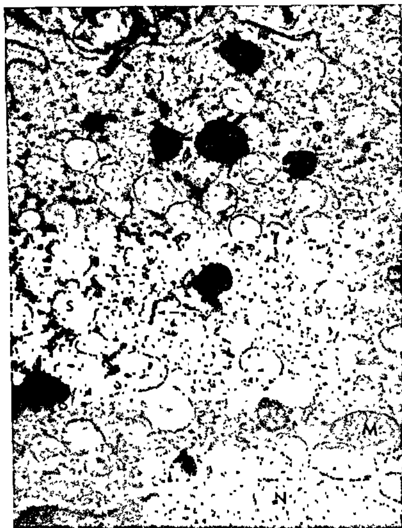
tissue or (2) interposition of an abnormal barrier between beta granules and blood A third possibility is a pathological change in the structure of granules in some diabetics. The similar appearance by light microscopy of beta granules [illegible] and diabetic man and in various animal species does not constitu [illegible]
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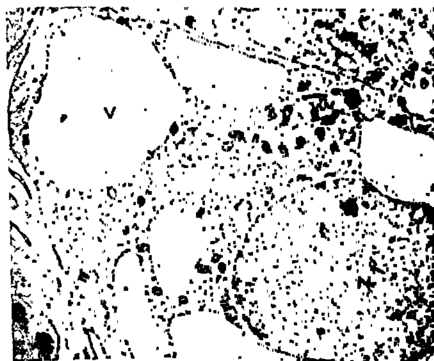
differences were complete[l] [illegible]
microscope Granules of [illegible]
because they are relatively [illegible]
only in the size of their consistently circular profiles.

Beta granules of the rat (FIGURE 6) are round and homogeneous, but in dogs (FIGURE 7) they have a rectangular profile. In cats (FIGURE 8) they have a central, dense, rhomboidal body embedded in a less dense homogeneous substance In chickens (FIGURE 9) the granules are distinctly crystalline and are composed of sheaves of needles. These examples of ultrastructural variations indicate that endogeneous insulin, or an intimately associated binding protein



nucleus ×24,000

limited by ergastoplasmic membranes; membranes of any type were absent around hydropic spaces in feline beta cells that contained glycogen and fluid (FIGURES 11 AND 12)



...a cell of a guinea pig after 13 days of ... are limited by a distinct membrane ... between the vacuoles A few alpha ... N = nucleus ×9000

... beta cells ... Orinase).* ... bly causes ... compilation of numerical indices of population densities of beta granules are being obtained before definite conclusions are drawn The changes produced in these preliminary studies are illustrated in FIGURES 7 and 13 Numerous rectangular granules are present in the control biopsy (FIGURE 7), but only a few are present 2 hours after an intravenous injection of Orinase (FIGURE 13). Membranous

* The Upjohn Company

Discussion of the Paper

ed
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diabetic subjects as an effective ... While such
ce of the
subjects,
with the effectively normal levels of plasma
insulin that have been reported in such subjects[1-3] How would Lacy and Hartroft interpret these findings in terms of their islet barrier hypothesis?

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cells or
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ic subject by increasing the
ells Normoglycemic levels
degree of release in the diabetic
in a diabetic, hyperglycemia (if, paradoxically, it could exist) would therefore stimulate the release of greater than normal amounts of insulin, that is sufficient ... ls of greater than nor-
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considerations are, of course, hypothetical, since we have no experimental condition that reproduces this type of diabetes in animals, neither have we, as yet, electron microscopic studies on diabetic subjects with a normal degree of beta granulation

granule saccules in these beta cells are empty but for small amounts of amorphous material

Summary

Frequently encountered pathological changes in islets of adult diabetics include hydropic or glycogenic vacuolation of beta cells, abnormal deposition of lipohyalin on or around the vessel walls, fibrosis and, more rarely, decreases in numbers of islets In 20 to 25 per cent of adult diabetics, islet abnormalities have not been demonstrated by techniques applied to man thus far. In all animal species studied to date, electron microscopy has shown that beta granules are separated from blood stream by a minimum of 5 spaces and 5 membranes, including walls of saccules in which they lie, plasmal membranes of cells, 2 basement membranes, capillary endothelium, and other structures. In theory, any of these spaces and membranes could be altered by the pathological deposition of either intracellular or extracellular fat, hyalin and collagen (singly or together), thereby threatening the normal degrees of freedom of interchange of glucose from blood to beta granule or insulin from granule to capillary lumen.

Diabetes has not yet been successfully produced in animals characterized by these kinds of abnormal insular deposits and by normal amounts of pancreatic insulin, which are features often present in diabetic man Therefore, electron microscopic study of this type of diabetes must depend upon the availability of biopsy material obtained from patients at laparotomy.

The ultrastructure of beta granules is uniquely species-specific for all animals

branching strands within the beta cells of cats injected intraperitoneally with large amounts of glucose solutions.

Preliminary studies of acute effects of intravenously administered Orinase on ultrastructure of beta cells in dogs suggest that degranulation occurs. Evaluation and interpretation of these findings await completion of additional experiments and collation of numerical incidences of population densities of beta granules

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jugular Nembutal anesthesia was used Blood samples were withdrawn at intervals from the inferior vena cava for the measurement of the blood glucose by the Nelson modification of the Somogyi method [1]

Following fixation in Bouin's fluid, specific granules were stained in 4-μ sections by the Gomori aldehyde-fuchsin method,[2] employing a chromotrope 2R-light green counterstain.

For demonstration of the Golgi apparatus, tissues were fixed in Champy-Kull fixative for 24 hours, washed for 24 hours, and treated with 2 per cent osmic acid for 8 hours at 56°C and for 10 days at 35°C They were then washed for 24 hours, dehydrated, embedded in paraffin, and sectioned at 4 μ Excess deposition of the oxide was removed with 0.125 per cent potassium permanganate followed by 3 per cent sodium bisulfite.

Results and Discussion

Identification of Cell Types

The distinction between alpha and beta cells was facilitated by the fact that, in the rat, the alpha cells are smaller and are almost completely restricted to the edges of the islets In Golgi preparations it is at once apparent that two cell types are present (FIGURE 1). The predominant cell is large and has a

and clear cytoplasm. These cells correspond, in their location and relative

counter-stained preparations only those cells that have been tentatively identified as alpha cells were so colored As a further assistance in identification, the islets of alloxan-diabetic rats were studied. Due to the destruction of the beta cells, the islets of these animals consist almost entirely of alpha cells, and in Golgi preparations, as would be expected, the islets were composed predominantly of the small cells with simple Golgi bodies, already tentatively identified as alpha cells (FIGURE 2).

Studies of Beta Cell Activity

show that elevation of the blood glucose level is a potent direct stimulus to the beta cells Of the many experiments bearing on this question, two are particularly conclusive Brown *et al* [3] infused high concentrations of glucose into part of the arterial supply to the pancreas and observed a lowering of the blood sugar and histological evidence of stimulation in the part of the pancreas that was infused Anderson and Long,[4] using the isolated, perfused rat pancreas, showed that the elevation of the blood glucose resulted in increased insulin levels in the perfusate. Since there is general agreement on this point, eleva-

USE OF THE GOLGI APPARATUS AS AN INDICATOR OF THE LEVEL OF ACTIVITY OF THE CELLS OF THE ISLETS OF LANGERHANS*

Adrienne Batts

Department of Physiology, University of California School of Medicine, San Francisco, Calif

The development of relatively sensitive methods for measuring insulin and glucagon in the circulating blood has been most valuable in estimating the level of islet function However, all of these methods leave much to be desired in precision, moreover, there are circumstances in which their application is not easy The alternative approach is to attempt to estimate the level of activity by histological means Conventionally, the activity has been judged on the basis of the amount of specific granular material in the cells, the size of the cells, and changes in the size and appearance of the nuclei The granule content may be very deceptive, however, since it obviously represents a balance between synthesis and release. It is possible, as will be shown, to show very definite depletion of granules not only in conditions of vigorous activity but also in conditions of extreme inactivity, moreover, the granule content may be unchanged under certain circumstances when other criteria indicate considerable reduction in activity It obviously would be desirable to have another, more reliable, means for determining the activity. A most likely candidate for this role is the Golgi apparatus, for it has been shown repeatedly that in a large variety of secreting tissues, both exocrin and endocrine, the Golgi apparatus becomes very elaborate during high levels of secretory activity and, on the other hand, is much reduced in size when this activity is low Widespread investigation of the exact nature and morphology of the Golgi apparatus in the living state is still in progress, and it appears quite likely that the ap-

regardless of its precise nature, responds in a reproducible way in a variety of functional states It can be shown that, in the islets, the Golgi apparatus responds quite rapidly and definitely to known stimuli, permitting exploration of function in a number of other conditions.

METHODS

Rats of the Long-Evans strain were employed exclusively During all experiments the animals were kept on a routine stock diet ad libitum unless otherwise specified. In some experiments alloxan-diabetic rats were employed. Diabetes was induced by the intravenous administration of 50 mg. of recrystallized alloxan per kilogram of body weight as a 5 per cent solution in saline following a 72-hour fast

In certain experiments glucose or insulin or both were infused intravenously through a catheter placed either in a tail vein or in a branch of the external

* The work reported in this paper was supported in part by Grant No A 1410 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda Md

jugular Nembutal anesthesia was used Blood samples were withdrawn at

tions by the Gomori aldehyde-fuchsin method,[2] employing a chromotrope 2R-light green counterstain

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Studies of Beta Cell Activity

was infused Anderson and Long,[4] using the isolated, perfused rat pancreas, showed that the elevation of the blood glucose resulted in increased insulin levels in the perfusate Since there is general agreement on this point, eleva-

tion of the blood glucose for varying periods of time was used as the first test

blood levels that were continuously above 350 mg. per cent. Animals were sacrificed at intervals from 1½ to 12 hours from the beginning of the infusion.



FIGURE 1 Islet of normal rat showing centrally located large cells with elaborate Golgi bodies (beta cells) and peripherally located small cells with small Golgi bodies (alpha cells) Osmic acid impregnation ×1100

Stains for specific granules revealed almost complete degranulation of the beta cells, a finding often described previously In the osmic acid preparation, the Golgi bodies of the beta cells of the experimental animals were somewhat larger and considerably more elaborate than those of the controls (FIGURES 3 and 4) This change was discernible at 1½ hours, well established at 4 hours, and quite marked at 12 hours. Presumably, more prolonged

observation that the beta cells of rats made diabetic through partial pancreatectomy show exceedingly hypertrophied Golgi bodies (FIGURE 5), presumably the result of very prolonged hyperglycemia

(2) *Hypoglycemia* Since hyperglycemia is a stimulus to the beta cells, it would be expected that the removal of this stimulus by reducing the blood sugar to levels below normal would result in great diminution of the activity of these cells This expectation is supported by the work of Zunz and La Barre[6] and

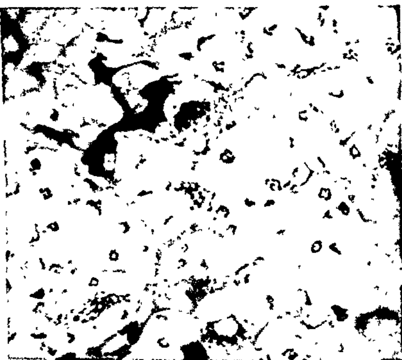


FIGURE 2 Islet of alloxan-diabetic rat Note that islet is composed almost entirely of cells with small Golgi bodies Osmic acid impregnation ×1100

also by the experiments of Bennett and Sutherland,[6] who transplanted normal pancreas into the necks of pancreatectomized dogs Bennett and Sutherland found that the blood sugar was restored to normal by this procedure following a highly reproducible curve of decline. They then observed that, in order for insulin infusions to duplicate this curve, it was necessary to reduce progressively the rate of infusion as the glucose level approached normal, indicating that the pancreas must also have reduced its rate of insulin secretion as the blood sugar fell One would therefore expect the islets to reflect in their appearance this reduction in activity during hypoglycemia

Rats were made hypoglycemic with insulin for short experiments of 4 hours'

duration and also for as long as 2 weeks. In the acute experiments, insulin was infused intravenously at 1.3 to 1.8 units/kg./hr., resulting in blood sugars of 40 mg. per cent or below. In the chronic experiments, 3 to 4 units of protamine zinc insulin were given subcutaneously every 12 hours for 2 weeks, resulting in blood sugars at autopsy of 37 mg. per cent or below. Four animals and their controls were used in each type of experiment. Stains for specific

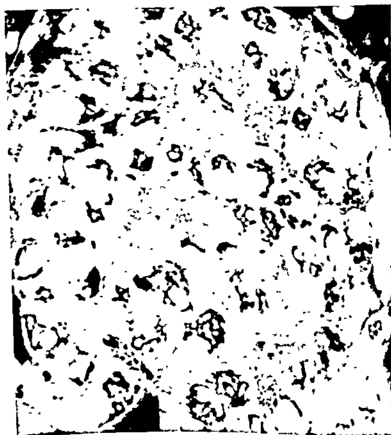


FIGURE 3. Islet of normal rat consisting almost entirely of beta cells. Compare with FIGURE 4. Osmic acid impregnation. X1100

granules in the acute experiments revealed a normal amount of granular material in the beta cells, but in the chronic experiments the beta cells were completely degranulated. In the acute experiments the Golgi bodies were somewhat condensed in appearance, while in the chronic experiments they were very much reduced in size and complexity (FIGURE 6).

It should be emphasized that in the two experimental conditions just described, the Golgi apparatus became hypertrophied during hyperglycemia and atrophic during hypoglycemia, thus apparently accurately reflecting the changes

in activity known to occur. On the other hand, in both these two quite opposite functional states the specific granules of the beta cells were almost completely depleted, in the one case presumably because insulin was released as rapidly as it was produced; in the other case, because the production of insulin was greatly depressed These findings emphasize the difficulties in interpreting changes in the granule content

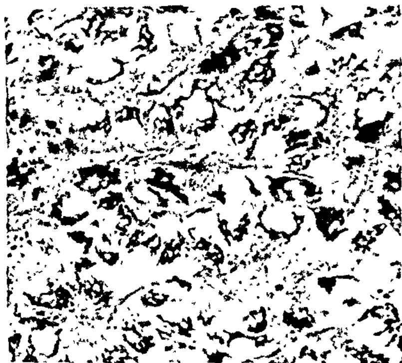


FIGURE 4 Islet of animal infused with glucose for 4 hours Note the increase in complexity of the beta cell Golgi bodies as compared to FIGURE 3 Osmic acid impregnation ×1100

The simplest explanation for the diminution in function during insulin-induced hypoglycemia would be that the lowered blood sugar has removed the necessary stimulus to activity However, the possibility that insulin itself may directly inhibit beta cell activity is suggested by the work of Hausberger

of them were subsequently infused with 1 unit of HGF-free insulin (Lilly) p

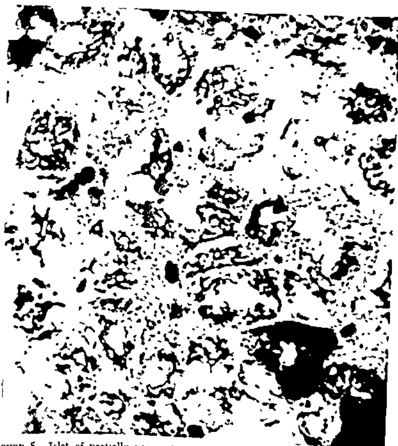


FIGURE 5 Islet of partially pancreatectomized, diabetic rat 60 days after operatio Note the extensive development of Golgi bodies in beta cells Osmic acid impregnatio ×1100

insulin and glucose showed extensive degranulation of the beta cells. Th Golgi bodies of the beta cells of those animals that received both glucose an insulin were noticeably more elaborate than those treated with insulin onl

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(3) *Influence of the pituitary.* Many years ago, a pancreotropic effect of the

pituitary on the islets was proposed by Anselmino and his co-workers[8, 9] on the basis of the finding that
hypertrophy of the islets
son and Young,[10] and has been shown [illegible]
arations of growth hormone.[11] If the pituitary exerts a stimulatory effect upon the islets, one would expect hypophysectomy to result in some degree of



FIGURE 6 Islet of rat treated with insulin for 2 weeks Note the reduction in complexity of Golgi bodies Compare with FIGURE 3 Osmic acid impregnation ×1100

hypofunction and consequent atrophy However, adequate estimations of islet mass have revealed only suggestions of such a change[11, 12] and the histology has been reported to be normal [13] It seemed worthwhile to reinvestigate this point

Twelve rats were hypophysectomized by the parapharyngeal approach at 40 days of age and were then placed in individual cages so that their food intake could be measured Twelve normal rats of the same age and sex were also placed in individual cages, and each was pair-fed with one of the hypophy-

sectomized rats. After 14 days both groups were autopsied, together with a group of ad libitum-fed controls, and the completeness of operation was checked by examination of the operative site Specific stains for granules showed is-

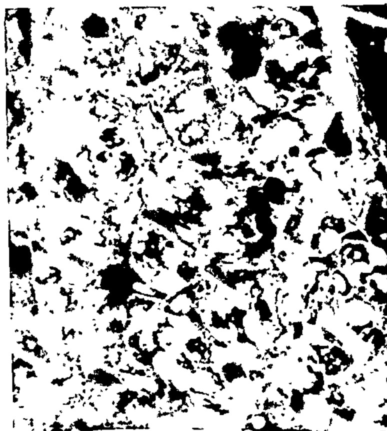


FIGURE 7 Islet of animal that was treated with insulin and glucose Beta cell Golgi bodies are more elaborate as compared with FIGURE 6 Osmic acid impregnation ×1100

ever, revealed a very marked reduction in the size and complexity of the Golgi bodies in the beta cells of the hypophysectomized rats, which was much more pronounced than the very moderate change seen in the [illegible]

[illegible] may have resulted from the absence of one or more stimulatory hormones Support for the concept that the beta cells are functioning at a low level in the hypophysec-

tomized animal is afforded by Randle,[14] who has found lowering of blood in-

[illegible]

when such a pancreas was transplanted into the neck of a diabetic dog, it re-

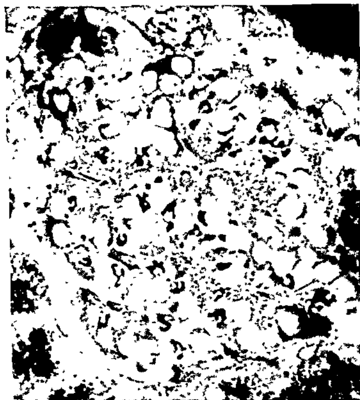


FIGURE 8 Islet of animal hypophysectomized 14 days previously Note the extreme reduction in complexity of Golgi bodies of beta cells Compare with FIGURE 3, an ad libitum-fed control, and with FIGURE 9, a pair-fed control Osmic acid impregnation ×1100

stored the blood sugar to normal in the same length of time as did the pancreas of a normal dog However, there is some suggestion in their data that the initial rate of decline in the blood sugar may have been slower when the pancreas of the hypophysectomized dog was used, even though the same final sugar level was attained

Most of the available evidence suggests that growth hormone stimulates the islets, probably directly, although in only a few cases has it been possible to observe this effect without a concomitant increase in blood sugar For ex-

ample, Randle[14] reported that the blood of cats treated with growth hormone contained increased insulin activity, but the blood sugars were also slightly elevated On the other hand, Anselmino and Hoffmann[15] long ago showed that pituitary extracts would produce a prompt decline in the blood sugar of the intact dog, but would produce only elevation in the blood sugar of dogs that had been previously pancreatectomized and allowed to recover thoroughly



FIGURE 9 Islet of normal rat whose food intake was restricted to that of animal whose islet is shown in FIGURE 8 Note moderate reduction in complexity of beta cell Golgi bodies. Osmic acid impregnation ×1100

from the effects of the operation. Moreover, Milman an[illegible]
served a [illegible]
or adrena[illegible]
terest to [illegible] of the beta cells by growth hormon could be detected histologically.

Four hypophysectomized rats, prepared as above, were treated for 4 day with 2.5 mg of growth hormone (prepared by the method of Wilhelmi *et al* [15] per day intraperitoneally. Another group of hypophysectomized rats wa

similarly treated, but their food intake was restricted to that of the untreated

[illegible]

However, their increased food intake makes interpretation of this experiment

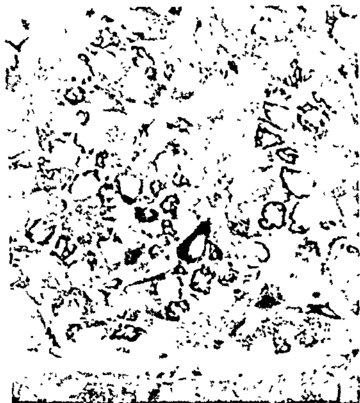


FIGURE 10 Islet of hypophysectomized animal treated for 4 days with growth hormone Note the return of Golgi bodies toward normal in appearance Osmic acid impregnation ×1100

difficult Therefore, an experiment was undertaken with normal rats under conditions that, it was hoped, would prevent either alterations in food intake

[illegible]

dose of insulin and also with 0 5 mg of purified growth hormone subcutaneously every 12 hours for 2 weeks At autopsy, the blood sugar values of the insulin-treated animals averaged 54 mg per cent, and those of the insulin- and growth

ample, Randle[14] reported that the blood of cats treated with growth hormone contained increased insulin activity, but the blood sugars were also slightly elevated On the other hand, Anselmino and Hoffmann[16] long ago showed that pituitary extracts would produce a prompt decline in the blood sugar of the intact dog, but would produce only elevation in the blood sugar of dogs that had been previously pancreatectomized and allowed to recover thoroughly



FIGURE 9 Islet of normal rat whose food intake was restricted to that of animal whose islet is shown in FIGURE 8 Note moderate reduction in complexity of beta cell Golgi bodies Osmic acid impregnation ×1100

from the effects of the operation Moreover, Milman and Russell[17] also observed a very marked decline in the blood sugar levels of hypophysectomized or adrenalectomized rats given growth hormone Therefore, it seemed of interest to determine whether stimulation of the beta cells by growth hormone could be detected histologically.

Four hypophysectomized rats prepared as abo[illegible]
with 2 5 mg of gro[illegible]
per day intraperit[illegible]

igation of the duct nor the destruction of the beta cells by alloxan produced any diminution in the amount of glucagon extractable from the pancreas,[19]

[illegible]

secreted in response to physiological stimuli. In cross-circulation experiments,



FIGURE 12. Islet of alloxan-diabetic rat treated with insulin for 2 weeks. Islet composed almost entirely of alpha cells. Compare with FIGURE 2. Alpha cell Golgi bodies unchanged by prolonged hypoglycemia. Osmic acid impregnation. ×1100

Foà *et al.*[22] have reported that pancreatic venous blood from a hypoglycemic dog contained a substance that induced hyperglycemia in a recipient animal, while mesenteric vein blood from the same animal did not. Moreover, Bornstein *et al.*[23] found that pancreatic venous blood from cats treated with growth

[illegible]

alpha cells with the expectation that the changes in function believed to occur would be reflected in their appearance. The effect of chronic insulin treatment

hormone-treated animals averaged 52 mg per cent. Study of the aldehyde fuchsin preparations revealed the usual degranulation in the insulin-group but, in the group treated with both hormones, the amount of beta cell granulation was restored toward normal. In the Golgi preparations, atrophy of the Golgi bodies was seen in the insulin-treated group as previously descr' ' but in the group treated with insulin and growth hormone the Golgi bodie

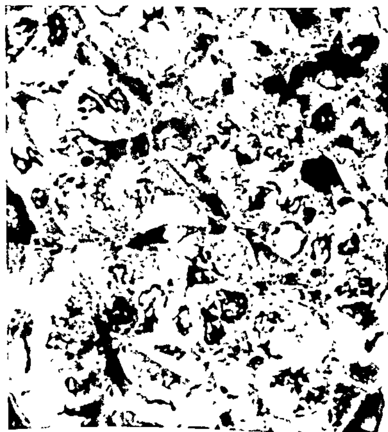


FIGURE 11. Animal treated with insulin and growth hormone for 2 weeks Compare with FIGURE 6 Note the increase in complexity of beta cell Golgi bodies compared to those of animal treated with insulin alone Osmic acid impregnation ×1100

were signific
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observed stin
ulation of the beta cells by the growth hormone itself rather than by any secondary effect on the blood sugar.

Studies of Alpha Cell Activity

Very strong evidence connects the alpha cells and the presence of extractable glucagon in the pancreas. Neither atrophy of the acinar tissue produced by

both groups of animals the granule content of the alpha cells was indistinguishable from the normal; furthermore, the Golgi bodies (FIGURE 12) appeared entirely unchanged

The effects of changes in pituitary function upon the histology of the alpha cells have been studied quite extensively Ferner *et al.*[28] have claimed that hypophysectomy resulted in reduced numbers of alpha cells, while growth hormone treatment increased their numbers However, it was later pointed out[13] that their staining method was unreliable, and further studies have revealed no change in the alpha cells following hypophysectomy, although very prolonged treatment with large doses of growth hormone resulted in an increase followed by a decrease in numbers. Therefore, the alpha cells were carefully

experiments outlined above are being repeated in rabbits On the other hand, *another possible explanation is afforded by the work of Sirek et al.*,[29] who have confirmed the finding that the administration of growth hormone causes the appearance in the pancreaticoduodenal vein blood of the dog of a material that induces hyperglycemia in recipient dogs However, this material was still present following growth hormone treatment of pancreatectomized dogs, moreover, its effect was blocked by pretreatment of the recipient animal with dihydroergotamine, a substance that blocks the effect of epinephrine, but not of glucagon.[30] These findings cast doubt upon the identity of the material found in the blood in the experiments cited above, and reopen the entire question of whether glucagon is actually secreted If it is not, the unchanging aspect of the alpha cells is quite understandable

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has been studied, with a variety of results. Latta and Harvey[25] treated mice with insulin for 75 days and observed no changes in the alpha cells. Hagemann[26] reported that chronic hypoglycemia increased the alpha-to-beta-cell ratio, but this finding could be accounted for on the basis of a reduction in the size and number of the beta cells. Recently, Hokfelt and Hultquist[27] have reported degranulation of the alpha cells in rabbits as a result of insulin treat-



FIGURE 13. Islet of animal hypophysectomized 14 days previously. Cells with light cytoplasm are alpha cells. Compare with FIGURE 1. Golgi bodies appear unchanged. Osmic acid impregnation. ×1100

ment. As a result of the divergent findings, studies of the Golgi bodies in the alpha cells of animals chronically treated with insulin were undertaken.

The alpha cells were studied in normal animals treated with insulin in the experiments described above. In no case did either the granul ... the Golgi bodies cha
Because of the r
loxan-diabetic ra
were maintained in a hypoglycemic state for 2 weeks as outlined above. In

MORPHOLOGIC REFLECTION OF INCREASING INSULINO-GENESIS DURING CHRONIC HYPERGLYCEMIA IN THE RABBIT

Bruno W Volk and Sydney S Lazarus

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In physiological studies of the effect on the blood sugar of various hyper-

Similarly, it has been observed that in some animal species there is gradual adaptation to the diabetogenic action of exogenously administered hyperglycemic agents Thus, the temporary diabetes induced by growth hormone or by adrenal steroids administered alone or together with glucagon is characterized by the gradual development of glycosuria, which then slowly declines only to become re-established on increasing the hormone dosage[4-11] The fact that

Material and Methods

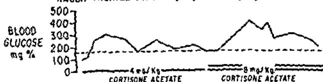
The study was carried out on 86 New Zealand white rabbits, of both sexes, weighing between 2500 to 4500 gm , divided into 4 groups Group 1 comprised 36 rabbits, each of which received cortisone acetate (4 mg /kg) intramuscularly daily during the first 21 days, and then 8 mg /kg daily for up to 60 days Group 2 consisted of 18 animals that received subcutaneously crystalline glucagon suspended in corn oil (1 mg /kg) 3 times daily, at 10 A M , 4.30 P M and 12 midnight and, in addition, cortisone acetate in aqueous suspension

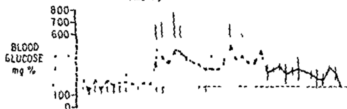
pancreatic tissue was placed immediately into Zenker formol solution (20 per cent) for histological study After paraffin embedding, the slides were

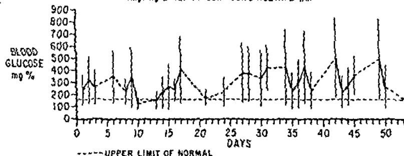
* Chas Pfizer & Co , Brooklyn, N Y

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nonvacuolated hyperplastic ductules were found that were intrainsular or contiguous with islets. In occasional instances, primitive beta cells were seen originating from this type of hyperplastic ductular epithelium (FIGURE 4).







stained by the following methods iron hematoxylin, phosphotungstic hematoxylin, modified Masson trichrome,[18] modified aldehyde-fuchsin trichrome,[19] a modification of Gomori's chrom alum hematoxylin,[20] and the periodic acid Schiff trichrome controlled by diastase digestion for glycogen identification[21]

Results

The untreated control rabbits showed no significant differences between the morning and afternoon blood sugar values, which varied between 93 and 158 mg per cent, a fasting blood sugar value higher than 160 mg. per cent was therefore considered a significant elevation. The animals that received large doses of cortisone alone showed, in general, 2 peaks of hyperglycemia: the initial peak blood sugar level was reached between the third and fifth days after starting treatment with cortisone (4 mg./kg). After that time the blood sugar tended to decline, reaching its nadir within 3 weeks. On doubling the dose of cortisone at 21 days, a secondary rise of the blood sugar level was usually obtained, which eventually again tended to decline to normal. With these cortisone dosages the maximum blood sugar levels varied up to 375 mg per cent (FIGURE 1, *top*)

The greatest degree and duration of hyperglycemia was developed in those rabbits treated simultaneously with cortisone and glucagon. In these animals the morning dose of glucagon produced a marked hyperglycemic response that was fairly well sustained until the afternoon The morning values were frequently within normal limits during the early stage of treatment; thereafter they increased to as much as 400 mg per cent. The afternoon values were elevated from the beginning This elevation became extreme as treatment continued, with occasional values reaching 830 mg per cent. After 6 weeks the morning glycemic levels were frequently less than they had been prior to that time, and tended to return to normal (FIGURE 1, *center*).

The animals treated with adrenaline plus cortisone showed intermittent hyperglycemia The morning blood sugar values varied between 82 and 120 mg per cent, and rarely exceeded upper normal levels. The afternoon values were markedly increased, and ranged up to 840 mg. per cent (FIGURE 1, *bottom*)

The sequential morphologic alterations of the pancreas of all hormonally treated animals were quite similar Within two days the degranulation of beta cells was observed which, concomitantly with increasing blood sugar levels, became very marked or even complete (FIGURE 2) The beta cells

eleventh days (FIGURE 3). Glycogenic vacuolization appeared first in ductular epithelium and then also in the beta cells, and its extent was related to the severity and duration of diabetes It is of interest that the rabbits treated with adrenaline plus cortisone, which developed only intermittent hyperglyce-

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hyperplastic, with irregular contours, and visual estimates of their volume showed that they were markedly increased over that seen in normal rabbits (FIGURE 7) Occasionally an acinar arrangement of beta cells was seen in which the cells surrounded a central lumen that contained Schiff-positive mate-



FIGURE 4 Pancreas of rabbit treated with glucagon and cortisone for 106 days, showing an intrainsular proliferating ductule (*arrows*) with a primitive beta cell (P) originating from it Periodic acid Schiff trichrome stain ×960

rial Additional morphologic changes in the pancreas unrelated to the chronic hyperglycemia were also found The animals that received glucagon and cortisone showed marked decrease of identifiable alpha cells, as described elsewhere [10] With the larger dosages of cortisone alone, but also to a lesser extent in the other two groups, there was occasional intraductular inspissation of secretion, ductular dilatation, and occasional pancreatic lobules that consisted of proliferating dilated ductules and dedifferentiated acinar tissue with comparatively intact islets

Discussion

Prolonged administration of diabetogenic hormones in the rabbit does not produce permanent diabetes The degree of diabetes wanes as treatment is

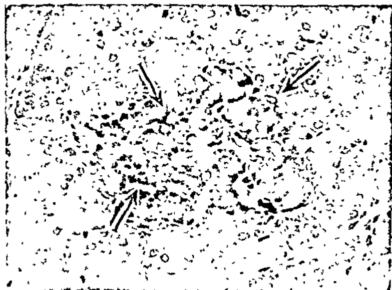


FIGURE 2 Islet of untreated rabbit showing well granulated beta cells (*arrows*) Aldehyde-fuchsin trichrome stain ×378

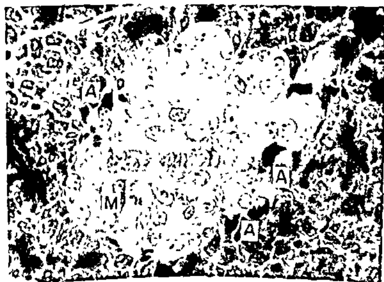


FIGURE 3. Pancreas of a rabbit treated with cortisone acetate (4 mg /kg) for 7 days There is a mitotic figure (M) and extensive B cell degranulation The alpha cells appear dark (A) Periodic acid Schiff trichrome stain ×500

(FIGURE 5) Occasional well-granulated beta cells were seen which were either intrainsular or intraductular (FIGURE 6) in location, despite the fact that the beta cells in the rest of the pancreas were in general extensively degranulated In the animals treated for the longest periods of time the islets were markedly hyperplastic, with irregular contours, and visual estimates of their volume

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FIGURE 4 Pancreas of rabbit treated with glucagon and cortisone for 106 days, showing an intrainsular proliferating ductule (*arrows*) with a primitive beta cell (P) originating from it Periodic acid Schiff trichrome stain ×960

rial Additional morphologic changes in the pancreas unrelated to the chronic hyperglycemia were also found The animals that received glucagon and cortisone showed marked decrease of identifiable alpha cells, as described elsewhere[10] With the larger dosages of cortisone alone, but also to a lesser extent in the other two groups, there was occasional intraductular inspissation of secretion, ductular dilatation, and occasional pancreatic lobules that consisted of proliferating dilated ductules and dedifferentiated acinar tissue with comparatively intact islets

Discussion

Prolonged administration of diabetogenic hormones in the rabbit does not produce permanent diabetes The degree of diabetes wanes as treatment is

prolonged, despite the maintenance of hormonal dosage. This decline of the hyperglycemia is thought to result from increased insulin release from the pancreas, which is reflected in the morphologic changes in it. Thus, soon after the initiation of hormonal treatment, there was degranulation and increased mitotic activity of beta cells. In the animals treated for prolonged periods of time there was marked islet hyperplasia, and it was possible to trace new beta cell formation from ductular epithelium.



FIGURE 5. Pancreas of same rabbit as in FIGURE 4, showing large young beta cells (B) with sparse aldehyde fuchsin positive granules. The adjacent older beta cells are smaller, degranulated, and have aldehyde fuchsin positive cell borders (*arrows*). Aldehyde fuchsin trichrome stain. ×1600

yperplasia,
, for hyper-
as we ... However, no unanimity of opinion exists as to the origin of newly glycemia. However, no unanimity of opinion exists as to the origin of newly formed beta cells. In the embryo, beta cells have been stated to develop either by differentiation from primitive ductular epithelium[26] or by transformation from acinar cells.[27] Under various conditions in postfetal life, neogenesis of beta cells has been claimed to occur from acinar cells and from other islet cell types as well as from ductular epithelium.[23, 28-32] The principal evidence for the former type of transformation has been either geographic contiguity, the supposed presence within the same cell of different types of intracellular granules, or an acinar arrangement of beta cells.[23, 29, 30] However, it is generally

iemselves, while primitive
n the study presented here,
, alpha cells, or delta cells
was found. This is in agreement with the observation that apparent acino-insular transformation, as well as the interchangeability of various islet cell types, is an artifact, either due to superimposition of two different cell types



or to a confusion of mitochondria with intracytoplasmic secretory granules[27]
lumen con-
s that these

self in morphologic changes of the beta cells indicative of increased insulinogenesis might

pensatory response Furthermore, only a very small percentage of individuals treated with adrenal steroids develops diabetes. It has also been shown that pronounced diabetes due to adrenocortical tumors or pheochromocytoma may be cured by surgery These observations suggest that in the human there is adaptation by the pancreas to diabetogenic stimuli, and diabetes may occur in man without causing damage to the pancreas sufficient to maintain the diabetic state after the offending lesion is removed.



FIGURE 7 Pancreas of rabbit treated with cortisone acetate (1 mg/kg) daily and 1 mg crystalline glucagon t.i.d. for 106 days, showing marked islet hyperplasia A newly formed islet is loosely attached to a hyperplastic ductule (*arrow*). Aldehyde fuchsin stain ×63

Summary

A study was conducted on the sequential morphologic changes in the pancreas of rabbits during administration of various diabetogenic hormones. Animals were treated either with large doses of cortisone alone or with subdiabetogenic dosages of cortisone together with glucagon or adrenaline The animals developed varying degrees of hyperglycemia but, in general, as treatment was prolonged, hyperglycemia tended to wane despite maintenance or even increase of the hormone dosage level.

The earliest morphologic change was degranulation of the beta cells, which gradually became complete. Within the first 3 to 11 days after treatment

ume of islet tissue. New beta cell formation was first evidenced by the appearance within ductules of large cells with opaque somewhat reticulated cytoplasm Subsequently, occasional cells were found with sparse, diffusely

genic agents and for the gradual diminution in diabetogenic effectiveness of the hormonal treatment

Acknowledgment

The microphotographs that illustrate this article were prepared by Herbert A Fischler

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STUDIES ON THE MECHANISM OF CAPTURE AND DEGRADATION OF INSULIN-I[131] BY THE CYCLICALLY PERFUSED RAT LIVER

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Since liver extracts have been shown to contain a potent insulin-degrading

served would reflect, not only the activity of the insulin proteolytic system, but also processes that govern the rate of entry of insulin into the cell, assuming an intracellular location for this system. Thus, failure to observe degradation under these conditions, despite the presence of insulinase in liver extracts, would argue strongly against a significant role of the liver in the degradation

fusion studies and present the results of more recent experiments designed to explore possible mechanisms of entry of iodoinsulin into the liver cell

Perfusion Experiments at 37°C.

FIGURE 1 is a schematic drawing of a recent modification of the perfusion apparatus used in many of these experiments[4] Heparinized whole rat blood was added to a round-bottomed flask reservoir-oxygenator As the flask was turned about its axis and slanted a few degrees from the horizontal, a film of blood was formed over its lower portion, permitting gas equilibration with an internal atmosphere of humidified 95 per cent oxygen and 5 per cent carbon dioxide After filtration, the blood was pumped through a portal vein cannula into the liver *in situ*, returning to the flask by means of a tube secured in the inferior vena cava above the diaphragm Retrograde leakage of blood was prevented by ligating the inferior vena cava between the liver and the right kidney A flow rate of 7.0 ml./min was maintained in most of these experi- flow for rat

the reservoir of the system described is depicted in FIGURE 2 The perfusate in the reservoir was sampled at the times indicated and the separated plasma treated with trichloro

plasma-

insulin,

TCA-sol

70 per cent of the iodoinsulin initially added was removed from the circulating perfusate We reported previously that the total quantity of TCA-precipitable radioactivity removed by the liver during the perfusions equaled the sum of

* Obtained from Abbott Laboratories, Oak Ridge, Tenn

the final plasma TCA-soluble radioactivity and the radioactivity accumulated by the cellular fraction of the blood[4] The latter was assumed to be derived from the products of iodoinsulin degradation, since the percentage of radioactivity found in the cellular fraction of blood with respect to the quantity of

... essentially all of the iodo-insulin that had been captured by the liver was degraded and its products returned to the circulation Of particular interest was the pronounced lag in the early appearance of TCA-soluble radioactivity when compared with the rapid disappearance of iodoinsulin (FIGURE 2) This discrepancy between the

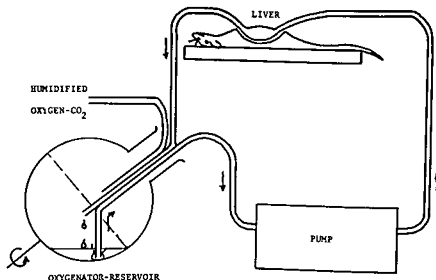


FIGURE 1 Schematic of organ perfusion apparatus See text for description

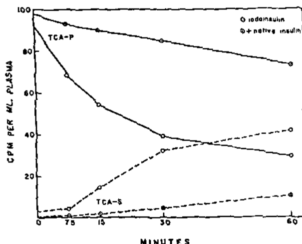
... egraded products, ... cumulated in the ... ion pointing to a separate ...

Evidence that at least a major fraction of iodoinsulin is handled in a manner similar to insulin is revealed in the competition that exists between native insulin and its iodinated derivative for one or more limiting steps in the degradative pathway (FIGURE 2). When iodoinsulin was diluted with increasing amounts of native insulin, increasing inhibition in both the rates of disappearance and degradation of iodoinsulin was observed[4] It would appear, then, that the liver cannot effectively distinguish native insulin from at least a major portion of iodoinsulin

The 60-min TCA-precipitable value in FIGURE 2 was shown, in perfusions carried out for extended periods of time, to be very close to the limit of removal

It was thus estimated that about 25 per cent of the iodoinsulin was relatively *unreactive* or resistant to capture by the liver. The iodoinsulin used in these experiments was estimated to have a mean iodine content of 1 atom per molecule of insulin (6000 M W.) At this level of iodination, we have shown previously that the diiodotyrosine (DIT) content is of the order of 15 per cent of

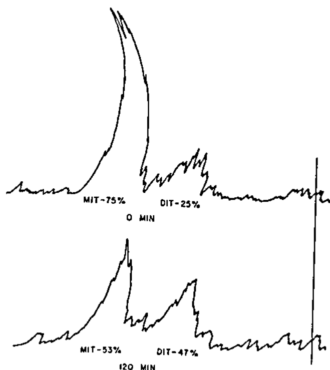
iodinated than others In order to determine whether this *heterogeneity* might have contributed to this resistant fraction, the relative DIT content of



the perfusate was determined after prolonged perfusion Plasma samples from the beginning and end of a 120-min perfusion were dialyzed to remove nonprotein radioactivity and then digested with pancreatin The digests were chromatographed and the radioactivity associated with MIT and DIT was measured for each sample (FIGURE 3) The significant increase in the proportion of DIT to MIT that became evident after all of the "active" TCA-precipitable radioactivity had been removed strongly suggests that the "inactive" fraction of iodoinsulin is composed of species having a higher than average iodine content It must be assumed, however, that the reiodination of other protein material had not occurred during the perfusion.

A calculation of the velocity constant for the hepatic removal of "active" iodoinsulin has been previously reported[4] For a more complete kinetic dis-

cussion the reader is referred to that communication, from which the following consi[illegible]
the l[illegible]
flow, [illegible]
time will cause essentially "instantaneous" concentration changes within the reservoir perfusate. The mean plasma TCA-precipitable values for iodoinsulin disappearance shown in FIGURE 2 were corrected by subtracting from each the



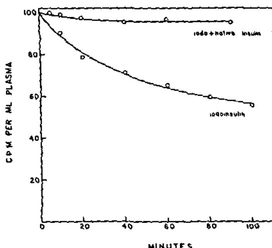
limiting or asymptotic TCA-precipitable value. From these corrected values a velocity constant of approximately 3 ml./min. was computed. Such a constant, which differs from the traditional constant $k = l/t$, is independent of changes in the volume of perfusate and, within limits, is also independent of the flow through liver. It may therefore be viewed as a quantitative approximation of the kinetics of the reaction occurring within the liver itself.

The percentage of iodoinsulin removed with respect to the amount delivered to the liver at any time, however, is related directly to the flow of perfusate. Having a velocity constant of removal of 3 ml./min. means that, of the iodoinsulin entering the liver, the amount removed will equal that contained in 3

ml. of the perfusate. Thus, since the established flow was 7 ml./min., three sevenths (40 per cent) of "active" iodoinsulin will be taken up during a single liver passage. This percentage extraction under simulated physiological conditions agrees very well with similar estimations made in intact rats.[6]

Cooled-Liver Perfusion Studies

then one should observe a substantial reduction in degradation by lowering the temperature of perfusion from 37° to nearly 0°C. With this in mind, a series

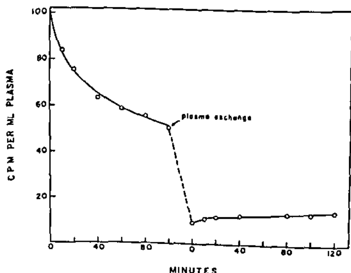


of experiments was conducted in a 3°C cold room. Livers from rats of the same sex, strain, and weight were perfused as before. The cell mass, however, was removed from the perfusate to reduce viscosity, and the plasma was equilibrated with air by constant stirring in a beaker surrounded with cracked ice.

the liver as TCA-precipitable material, virtually none was bound to the tubing or the glass reservoir. When a large amount of native insulin was dissolved in the iodoinsulin solution (FIGURE 4) the disappearance of the latter was nearly

abolished, thus establishing the existence of a saturable site of removal and eliminating the possibility that iodoinsulin nonspecifically diffused into fluid spaces of the liver

The essential irreversibility of this disappearance is indicated in FIGURE 5. As in earlier experiments, iodoinsulin was removed by the liver during 100 min of perfusion Immediately thereafter, the plasma in the reservoir was exchanged for fresh plasma After 5 min of circulation to permit equilibration, a zero-time sample was taken, leaving a circulating volume equal to the initial volume Note the barely perceptible increase in TCA-precipitable radioactivity during the second half of this experiment The implication that the process of removal was one of strong binding to the liver was strengthened by



the results of the following experiment. At the termination of a separate 100-min. cold perfusion, residual unbound iodoinsulin was eluted from the liver by perfusing with 0 25 M sucrose A portion of the liver was then minced and homogenized in an 0.25-M sucrose medium containing native insulin (40 μg/ [illegible], should iodoinsulin be- [illegible] main unbound. After [illegible] action, when compared [illegible] the total radioactivity, one half of which was TCA-soluble. Inasmuch as the presence of native insulin should hinder or even reverse binding, the fact that nearly all of the captured iodoinsulin sedimented under these conditions establishes the existence of stable binding between iodoinsulin and a particulate fraction of the liver cell.

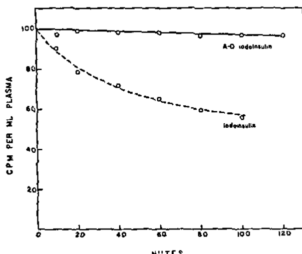
Failure to detect any degradation during the cold perfusions was surprising, since or [illegible] of liver homogenate experiments a sharp [illegible] ture [illegible] insulin at 0°C. was tested. A 100-min cold perfusion [illegible] at its conclusion, unbound iodoinsulin was eluted from the liver with saline.

TABLE 1

DEGRADATI[illegible]

Per Cent TCA [illegible]

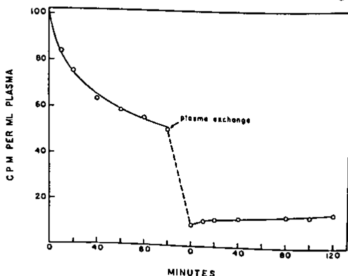
Incubation temperature	Slice	Homogenate
0°C	3	22 1
9°C	9 8	50 2
25°C	23 3	89 2



A segment of the liver was then homogenized in 0 1 M Tris buffer, pH 7 6, yielding a 10 per cent homogenate. Portions of this homogenate were incubated [illegible] 25°C. Slices of the same liver, mois[illegible] After 60 min the incubations were [illegible] together with homogenization of the slices [illegible] for each sample, during the incubation; the results [illegible] cent of the bound iodoinsulin was degraded during the 0°C. homogenate incuba-

abolished, thus establishing the existence of a saturable site of removal and eliminating the possibility that iodoinsulin nonspecifically diffused into fluid spaces of the liver

The essential irreversibility of this disappearance is indicated in FIGURE 5 As in earlier experiments, iodoinsulin was removed by the liver during 100 min. of perfusion Immediately thereafter, the plasma in the reservoir was exchanged for fresh plasma After 5 min of circulation to permit equilibration, a zero-time sample was taken, leaving a circulating volume equal to the initial volume Note the barely perceptible increase in TCA-precipitable radioactivity during the second half of this experiment. The implication that the process of removal was one of strong binding to the liver was strengthened by



homogenized in an 0.25-M sucrose medium containing native insulin (40 μg/ml.). The concentration of native insulin was such that, should iodoinsulin become unbound during homogenization, it would likely remain unbound. After centrifuging at 100,000 g for 30 min the supernatant fraction, when compared with the whole homogenate, carried only 11 per cent of the total radioactivity, one half of which was TCA-soluble. Inasmuch as the presence of native insulin should hinder or even reverse binding, the fact that nearly all of the captured iodoinsulin sedimented under these conditions establishes the existence of stable binding between iodoinsulin and a particulate fraction of the liver cell.

the cold was not followed by degradation, even after 4 hours of continuous erfusion In spite of the complete inhibition of degradation observed in the ıtact liver, the proteolytic system was active since an appreciable fraction of ound iodoinsulin was subsequently degraded at 0°C. after the liver was homogıized (TABLE 1). As mentioned earlier, perhaps an essential component ecessary for degradation remained physically isolated in the intact cooled

cheme might then be the rate-limiting transfer process postulated for the 37°C. erfusions

Summary

Iodoinsulin was shown to be rapidly degraded by the isolated, cyclically perfused rat liver At a rate of blood flow that was estimated to approximate

that cooling the intact liver and perfusate to 3°C completely abolished iodo-

Acknowledgments

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liver where proteolytic activity, as noted in the above experiment, was only partially inhibited by cooling, it is reasonable to evoke the idea that an essential element was physically separated from the other components of the degradative system. Such an element might be an enzyme, cofactor, or iodoinsulin itself. If iodoinsulin is the isolated component and it is further assumed that the site of binding is on or within the cell where degradation occurs, then one may postulate the existence of an insulin transport or transfer mechanism which may be more sensitive to the effects of cooling than is the degradative system itself.

Some degree of selectivity in the binding of iodoinsulin is indicated by its very removal from plasma, a medium abundant in protein. Furthermore, iodoinsulin th...
(FIGURE 6)
insulin by ch...
linkages.[6] Finally, no competition was noted between iodoinsulin and glucagon or prolactin for hepatic binding in the cold when the latter were added to the perfusate in amounts comparable to native insulin as shown in FIGURE 4.

Comments

It will be recalled that during the first 15 min. of perfusion at 37°C. a rapid disappearance of iodoinsulin was observed, while the products of degradation increased only very little (FIGURE 2). It was shown subsequently that this initial rate of iodoinsulin disappearance could be markedly inhibited by adding to the perfusate unlabeled native insulin, an observation that lead to the conclusion that a nonspecific diffusion of iodoinsulin into fluid compartments of the liver contributed little, if at all, to its disappearance. Such a dichotomy in rates obviously results in the hepatic accumulation of iodoinsulin or its products. It has been reported that, after administering iodoinsulin to intact rats, most of the radioactivity in livers removed within 15 min. after injection was precipitable with TCA.[7] Furthermore, the hepatic concentration of this material was several times higher than one would expect from simple diffusion alone.[7] Thus, this initial discrepancy in rates and the concomitant accumula-

it is not readily apparent why iodoinsulin would be permitted to accumulate unless its site of capture were remote from the degrading ...
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of capture to the degradative system.

The results of the cooled-liver perfusion experiments are of interest to this discussion since they outline more graphically the hypothesis just presented. The selective binding of iodoinsulin that was observed during liver perfusions

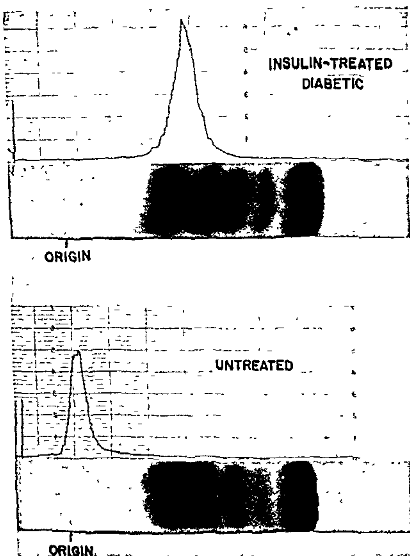


FIGURE 1 Paper electrophoretograms of insulin I^{131} in the serum of an insulin-treated diabetic subject (*top*) and in the plasma of an untreated control subject (*bottom*), together with radioactive scans of the paper strips

RECENT STUDIES ON INSULIN-BINDING ANTIBODIES

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Radioisotope Service, Veterans Administration Hospital, Bronx, N. Y

Insulin-binding antibodies uniformly appear in the sera of human subje after 1 to 3 months of continuous insulin therapy.[1] Since the antigen-antibc complexes are not precipitable and the maximum insulin-binding capacity generally only of the order of 200 to 300 mμg /ml. serum, detection of i antibody requires special techniques In our experience, the use of I^{131}-labe insulin and paper chromatography[1] or electrophoresis[1, 2] has proved m expedient for quantitative as well as qualitative analysis of insulin-antiser mixtures When present in low concentrations, free insulin-I^{131} adsorbs firm to Whatman 3 MM ...

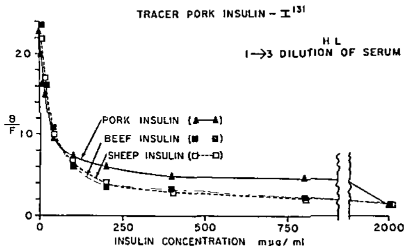
from the blood stream[...] and for the protection of insulin against the degrad tive action of liver insulinase[9] and, probably, of other insulin-degrading e zymes, as well When present in high concentrations, the insulin-bindi antibody is associated with manifest clinical insulin resistance. Whereas tl insulin-binding capacities of sera from most insulin-treated patients do n generally exceed 10 U /l serum,[1, 10-12] ...
bind from 60 to 500 U /l and mor
insulin requirements depends on a
concentration of antibody These factors include the rates of formation an dissociation of insulin-antibody complexes, which are in part determined b the energies of reaction between insulin and antibodies and the rate of im munological elimination of complexes[13] The reversibility of insulin-antibod complex formation[1, 9-13] may result in delayed insulin reactions even sever days after large amounts of insulin have been administered during the trea ment of insulin resistance associated with high antibody concentrations.[11]

At least two types of insulin-antibody complexes are formed; their rates c dissociation differ by a factor of 25 to 100.[10-13] The standard free-energ changes involved in formation of these complexes (minus 10 to 11 kcal./mo for one and minus 13 to 14 kcal /mol for the other[13, 15]) are considerably greate than those reported for most other antigen-antibody reactions[16] The pos tive entropy changes in these reactions (ΔS^0 = 25–30 entropy units/mol) have been interpreted in other antigen-antibody systems as being due to tl release of bound water molecules at the sites of reaction.[17]

In general, equilibrium constants were found to be signific...

In the light of this fact, the high energy of interaction of sheep insulin with antibody has been interpreted[19] as indicating the important contribution of van der Waals' attractive forces (in consequence of close spatial complementarity of antibody and antigen) to the stability of the antigen-antibody complex. In support of this conclusion is the observation that the equilibrium constants are not significantly dependent on the ionic strength of the solutions within wide limits of the latter.[4]

The specific quantitative relationship between the concentration of insulin present in insulin-antiserum mixtures and the percentage of insulin-I^{131} that becomes bound to antibody has been used to advantage in the development of an immunoassay for insulin.[21] However, the assay is necessarily specific for



individual animal insulins and not for the hormonal activity of insulin and therefore is not affected by the presence of other insulin antagonists. Thus, this method can assay the concentration of any insulin (for example, beef,

available but, if the insulin content is known, even a crude preparation will suffice. The method is briefly as follows.

is added also to unknown samples. Following incubation of all solutions with the same concentration of antiserum, the mixtures are analyzed for their content of bound insulin-I^{131} (B) and free insulin-I^{131} (F). The ratio B/F is then

sera from nonresistant subj
sulin-resistant patients (fig
to indicate that some loss i
when antibody synthesis is

Recent studies have show...
in the energy of reaction with insulins of different animal species. Beef and sheep insulins are generally bound much more strongly than pork and horse insulins,[18, 19] as evidenced not only by greater direct binding of the labeled insulins but also by more effective competitive inhibition on cross reaction

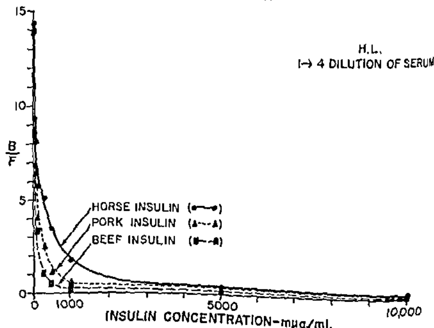
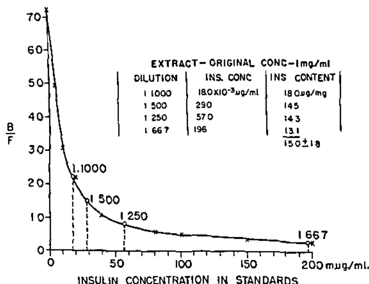


FIGURE 2. Ratio of bound to free beef insulin-I131 (tracer concentrations) as a function of concentration of unlabeled horse, pork, and beef insulins.

(FIGURES 2 and 3). Inasmuch as these various animal insulins differ in amino acid sequence only in the 8, 9, and 10 residues of the A chains,[20] it would seem that this region not only plays an important part in the reaction with antibody but also represents a site of antigenicity. It should not escape attention that the similarly reacting animal insulins contain the same amino acids in the 8 and 10 positions
and threonine at
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special interest that ...
pork insulin, and that the *R* portions of the 8, 9, 10 residues (alanine, glycine, and valine) of sheep insulin *A* chain contain no permanently charged groups

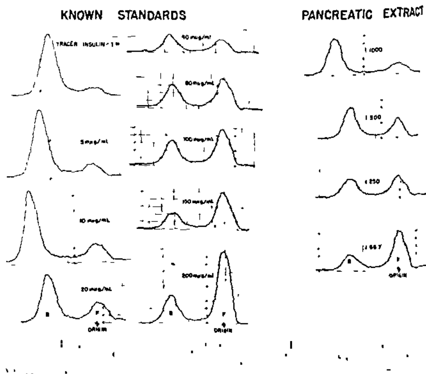
as low as 5 or 10 microunits/ml, but any given reduction in B/F ratio for beef (or pork, or horse, or sheep) insulin-I^{131} requires from 25 to 100 times as much human as animal insulin. Therefore, human antisera produced against beef or pork insulins are not very satisfactory for assay of human insulin at the concentrations that usually obtain in blood. However, human insulin has

able in quantities sufficient for standardization of different antisera in many



laboratories, we
pig antiserum th
human insulin
tors with such standardized antisera, toge
curves. The study of endogenous insulin concentrations in man may thus be expedited
sufficiently
or 1:200,
the low insulin concentrations present in the blood stream. From present indications it appears that this will be quite feasible, but several months will elapse before standardized sera will be available. Another difficulty that may

plotted as a function of the insulin concentration present in the known solutions to provide a "standard curve" By reference to the standard curve the insulin concentration in unknown samples is readily determined from the observed B/F values If the unknown solutions are assayed at several dilutions concentrations, the B/F values fall along different regions of the standard curve and the precision of the determinations is thereby increased. The method and results obtained in the assay of a crude extract of beef pancreas are illustrated in FIGURES 4 and 5 The values obtained were in good agreement with the results of assays by the mouse convulsion test.[21] * Since the slope of the



curve of B/F versus insulin concentration decreases with increase in insulin concentration, the change in B/F for any given percentage increment in insulin will be greater as the initial insulin concentration is lower. Furthermore, since the equilibrium constants of the react- ... and human anti- ... precision and the ... tures are allowed ... at 37°C. for several hours

Selected human antisera permit the detection of beef insulin concentrations

* The mouse convulsions assays were performed by Reuben Schucher of the Montreal Jewish Hospital, Montreal, Que, Canada

INSULIN-STIMULATED GLYCOGEN FORMATION IN RAT DIAPHRAGM*

Joseph Larner, Carlos Villar Palasi,† David J. Richman
Department of Pharmacology, Western Reserve University School of Medicine, Cleveland, Ohio

Introduction

[illegible] synthesis in isolated rat [illegible] ll and Hamman[1] Brown [illegible] ation period, about 75 per cent of the increased glucose uptake in the presence of insulin could be accounted for as tissue glycogen. In the present report it is shown that, after a 10-min incubation period at 38°C, more than 90 per cent of the increased glucose uptake in the presence of insulin could be accounted for as tissue glycogen, the major increase being in a *perchloric acid-soluble glycogen fraction*

Analyses of tissue acid-soluble phosphate intermediates, including inorganic phosphate, creatine phosphate, adenosine diphosphate (ADP), and adenosine triphosphate (ATP) showed no difference between control and insulin-treated diaphragms A small but statistically significant increase in hexose-6-phosphate content of the insulin-stimulated diaphragms was observed at 2 medium glucose levels

Glycogen synthesis occurred *with ratios of inorganic phosphate to glucose-1-phosphate* that would markedly favor glycogen degradation by phosphorylase as studied in soluble systems Enzyme studies in broken-cell preparations indicate the presence in skeletal muscle of 2 enzymes, uridine diphosphate [illegible]

Materials and Methods

Twelve male Wistar rats weighing between 70 and 100 gm after an 18 to 24-hour fast were used for each experiment Incubations were carried out in Gey and Gey bicarbonate buffer[6] and gassed with 95 per cent O_2 and 5 per cent CO_2 Vessels were shaken at 140 cycles per minute in the metabolic shaker

Prior to the 10-min experimental period at 38°C, 2 pre-experimental pro- [illegible] to allow insulin fixation to muscle cells to occur Following the second period, the experiment was run for 10 min at 38°C. Amorphous insulin (0 1 U/ml) [illegible]

be mentioned only briefly here is that of producing the required high-specific activity insulin-I[131] free of excessive amounts of irradiation-damaged components.[1, 22] However, to a large extent this problem has been solved by judicious attention to procedures that limit such damage[23] and in any event can be circumvented by simultaneously running the mixtures with control sera to correct for nonspecific plasma protein-binding of damaged insulin-I[131].[11]

Acknowledgments

We are indebted to O K Behrens, C W. Pettinga, H. Neurath, F. Tietze, J Field, and L C Craig for gifts of the various insulins used in these studies

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ion at 38°C are presented in TABLE 1. Glucose uptake values are reported n the units mg/gm/10 min When expressed in the more conventional

More than 90 per cent of the increased glucose uptake in the presence of nsulin could be accounted for as increased tissue glycogen at medium glucose concentrations of 140 and 280 mg/100 ml At both concentrations the major ncrease noted was in the acid-soluble glycogen fraction [11] At 280 mg/100

TABLE 1
MEDIUM GLUCOSE UPTAKE AND GLYCOGEN FORMATION BY CONTROL AND INSULIN-STIMULATED RAT DIAPHRAGMS
(Medium Glucose Uptake)

Medium glucose concentration mg/100 ml	Control mg/gm/10 min	Insulin mg/gm/10 min	Mean difference mg/gm/10 min.
140	1 43 (13)*	1 82 (13)	0 39 ± 0 027 <0 001†
280	1 95 (13)	2 41 (13)	0 46 ± 0 049 <0 001†

Tissue Glycogen Content

Perchloric acid soluble			Residual			
Control mg/gm	Insulin mg/gm	Mean difference mg/gm/10 min	Control mg/gm.	Insulin mg/gm	Mean difference mg/gm/10 min	Glycogen yield per centages
0 814 (10)	1 172 (10)	0 358 ±0 053 <0 001†	1 641 (9)	1 687 (9)	0 046 ±0 031 <0 25 >0 20†	92
0 774 (8)	1 073 (8)	0 299 ±0 062 <0 01†	1 438 (9)	1 560 (9)	0 122 ±0 038 <0 02 >0 01†	92

* Number of experiments indicated by the figure in parenthesis Each experiment is the mean of 6 control and 6 insulin treated vessels, each vessel containing 2 hemidiaphragms
† p

periods on a balance basis, the increased glucose uptake in the presence of insulin in this preparation can be accounted for to an almost quantitative extent as increased tissue glycogen

Acid-soluble phosphates Analyses of acid-soluble phosphate classes are presented in TABLE 2 These analyses were done on neutralized perchlorate filtrates from diaphragms incubated at a medium glucose concentration of 140 mg/100 ml *Acid-labile phosphate represents 37.9 to 41 4 per cent of the* total acid-soluble phosphate. These values are in agreement with values reported for heart,[12] using a rapid-freezing technique for tissue fixation No

was freshly prepared each day prior to use. Following the experimental period, beakers were rapidly chilled at 0°C.; then the tissue was removed quickly washed, blotted, and frozen in solid-liquid isopentane prechilled in liquid nitrogen Medium samples were deproteinized by the Ba-Zn method for glucose analysis The experiment was carried through with pairs of mals according to a timed schedule

Tissue Extraction

Twelve control and 12 insulin-treated hemidiaphragms from paired ves were pooled and mechanically reduced to a fine powder in a fitted stai

ing for 5 min with a mechanically driven plastic (Teflon) pestle Extra. were combined, brought to a known volume, and neutralized by adding solid $KHCO_3$ to neutral *p*H, $KClO_4$ was removed by centrifugation, and the supernatant fluid was used for the various analytical techniques employed

Analytical

Medium glucose uptake was determined as reducing power by the method of Nelson[7] Acid-labile phosphate was determined after a 30-min hydrolysis in NH_2SO_4 at 100°C Creatine phosphate was estimated by the method of Lowry and Lopez[8] with acetate buffer at *p*H 5 0. Anthrone analysis was done in a final volume of 3 ml with a concentration of H_2SO_4 of 66 per cent

Hexose phosphates were estimated microenzymatically in lyophilized neutralized perchlorate extracts of diaphragm or in lyophilized Dowex-2 column fractions For each determination, a major portion of the perchlorate extract was disolved in a small aliquot of water Glucose-6-phosphate dehydrogenase and crystalline phosphoglucomutase in the presence of added glucose-1, 6 diphosphate were used Since the glucose-6-phosphate dehydrogenase preparation is contaminated by isomerase, no distinction was made between glucose 6 phosphate and fructose-6-pl
absence of hexokinase and
dehydrogenase preparation[9]
for the recovery of added gl
occasional addition at the end of the determination

Residual glycogen was estimated after digestion of insoluble diaphragm residue with 1 to 2 ml of 10 per cent KOH at 100°C for 60 to 90 min. Glycogen was precipitated with 66 per cent ethanol Precipitates were washed twice with 66 per cent ethanol before dissolving in a known volume of water

Perchloric acid-soluble glycogen was estimat...
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of estimation of acid-soluble glycogen.

Results

Glucose uptake and glycogen synthesis Balance studies relating insulin stimulated glucose uptake to glycogen synthesis following the 10-min incub

diaphragm by Sacks.[14] Calculations show that, in order completely to resynthesize the ATP required for the insulin-stimulated glycogen formation, 6 per cent of the increased glucose uptake would be required, assuming an average P/O ratio of 3 and a requirement of 2 ATPs per mole of glucose polymerized

shown, oxidative phosphorylation can be made dependent in an almost absolute sense on the availability of ADP as phosphate acceptor for ATP resynthesis[15]

Hexose phosphates Hexose-6-phosphate and glucose-1-phosphate contents of control and insulin-treated diaphragms after 10-min incubations at 38°C have been measured At a medium glucose concentration of 140 mg./100 ml, mean hexose-6-phosphate contents were 0 109 and 0 136 μmole/gm. for control and for insulin-treated diaphragms, respectively (TABLE 4) The small increase noted (0 027 ± 0 008) was statistically significant, mean glucose-1-

TABLE 4
HEXOSE MONOPHOSPHATE CONTENTS OF CONTROL AND INSULIN-STIMULATED RAT DIAPHRAGMS

Medium glucose concentration mg./100 ml.	Glucose 1 phosphate			Hexose-6-phosphate			
	Control μmoles/gm.	Insulin μmoles/gm	Difference μmoles/gm	Control μmoles/gm	Insulin μmoles/gm	Mean difference μmoles/gm	p
140	0 034 (6)	0 028 (6)	—	0 109 (7)	0 136 (7)	0 027 ±0 008	<0 02 >0 01
280	0 048 (8)	0 050 (8)	—	0 169 (8)	0 212 (8)	0 043 ±0 014	0 02

Ratio P/G 1 − P (8 49/0 028) = 250 (8 55/0 028) = 305

phosphate contents were 0 034 and 0 028 μmole/gm, respectively Glucose-1-phosphate levels are considerably lower than hexose-6-phosphate, and in the direction of phosphoglucomutase equilibrium. There was no significant difference between control and insulin-treated tissue contents of glucose-1-phosphate

Similar findings have been made at a medium glucose concentration of 280 mg./100 ml Mean hexose-6-phosphate contents were 0.169 and 0 212 μmole/gm for control and insulin-treated tissue, respectively The small difference observed (0 043 ± 0 014) was again statistically significant Mean glucose-1-phosphate contents were 0 048 and 0 050 μmole/gm, respectively, no significant difference being observed in this fraction

The increase noted in the hexose-6-phosphate fraction of the insulin-treated diaphragm at the 2 medium glucose concentrations is compatible with an insulin-stimulated conversion of medium glucose to tissue hexose-6-phosphate

The absence of an increase in the glucose-1-phosphate fraction that might be expected to increase concomitantly with the hexose-6-phosphate fraction may be explained in one of two possible ways

(1) The expected increase is counterbalanced by removal of glucose-1-phos-

significant differences were noted in inorganic phosphate or creatine phosphate. With an insulin-stimulated glycogen synthesis of 2 μmoles/gram/10 mins no decrease in acid-labile phosphate was noted; on the contrary, a small but statistically significant increase was observed.

ADP and ATP That this increase was not due to an increase in ADP ATP was shown by analysis of these compounds after separation on Do chloride ion exchange resins. Analyses were carried out by 2 independent

TABLE 2
ACID-SOLUBLE PHOSPHATE ANALYSIS OF CONTROL AND INSULIN-STIMULATED RAT DIAPHRAGMS

	Inorganic phosphate		Phosphocreatine		Acid labile phosphate	
	Control μmoles/gm	Insulin μmoles/gm	Control μmoles/gm	Insulin μmoles/gm	Control μmoles/gm	Insulin μmoles/gm
	7 36	9 71	5 02	5 07	7.73	8 07
	8 60	8 06	5 22	6 14	10 40	11 80
	9 87	7 46			9 87	10 50
	9 76	9 82	3 76	2 42	7 05	8 66
	8 43	9 12	4 74	4.63	8 35	9 10
	6 91	7 10	5.00	3 87	7 75	7 76
Mean	8 49	8 55	4 75	4 43	8 53	9 32
Mean difference					0 79 ± 0 25	
p	0 5		<0 5 >0 4		0 025	

TABLE 3
ADP AND ATP CONTENTS OF CONTROL AND INSULIN-STIMULATED RAT DIAPHRAGMS

	ADP (as adenine)		ATP (as adenine)	
	Control μmoles/gm	Insulin μmoles/gm	Control μmoles/gm	Insulin μmoles/gm
	1 49	1 31	2 39	2.81
	0 68	0 85	1 64	1 65
	0 75	0 78	1 74	1.56
	0 93	0 87	2 25	2 36
Mean	0 96	0 95	2 00	2 09

measurements: namely, ultraviolet absorption at 260 μ, and radioactivity using P^{32}. For these experiments animals were preinjected with P^{32}-inorganic phosphate (2 mc. per rat) 18 to 24 hours prior to the experiment

TABLE 3 presents the ultraviolet analytical data for ADP and ATP from such experiments As may be noted, ADP and ATP levels did not differ significantly between control and insulin-treated diaphragms Rye Alertson *et al* [13] have recently reported similar findings after 30-min incubation periods

These findings are in agreement with the increased turnover of high-energy phosphate bonds of creatine phosphate and ATP observed in insulin-stimulated

Calculations show that, in order completely to resyn-

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Hexose phosphates Hexose-6-phosphate and glucose-1-phosphate contents of control and insulin-treated diaphragms after 10-min incubations at 38°C have been measured At a medium glucose concentration of 140 mg./100 ml, mean hexose-6-phosphate contents were 0 109 and 0 136 μmole/gm. for control and for insulin-treated diaphragms, respectively (TABLE 4). The small increase noted (0 027 ± 0 008) was statistically significant, mean glucose-1-

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phosphate contents were 0 034 and 0 028 μmole/gm, respectively Glucose-1-phosphate levels are considerably lower than hexose-6-phosphate, and in the direction of phosphoglucomutase equilibrium There was *no significant* difference between control and insulin-treated tissue contents of glucose-1-phosphate

Similar findings have been made at a medium glucose concentration of 280 mg./100 ml Mean hexose-6-phosphate contents were 0 169 and 0 212 μmole/gm for control and insulin-treated tissue, respectively. The small difference

The increase noted in the hexose-6-phosphate fraction of the insulin-treated

be expected to increase concomitantly with the hexose-6-phosphate fraction may be explained in one of two possible ways.

(1) The expected increase is counterbalanced by removal of glucose-1-phos-

phate (presumably to glycogen) through a kinetically rapid reaction, possibly such as the UDPG pyrophosphorylase reaction (see below).

(2) A specific insulin-stimulated removal of glucose-1-phosphate that would lead to the concept of a double effect, but not necessarily double action of insulin in diaphragm.

Ratios of inorganic phosphate to glucose-1-phosphate. In view of the low levels of glucose-1-phosphate observed under these conditions, it was of interest to express these levels in terms of ratios of inorganic phosphate to glucose-1 phosphate. Through the extensive work of Cori[16] and of Hanes[17] it is well recognized that whether synthesis or degradation of glycogen by phosphorylase will occur depends on the value of this ratio, which at equilibrium has a value of 3 at neutral pH Ratios higher than 3 result in glycogen degradation, while ratios lower than 3 result in glycogen synthesis. The concentrations of reactive sites (outer chains) of the glycogen primer do not ordinarily enter into the expression, since their size and not their number changes with lengthening of

ratios observed in these experiments at 250 and 305, respectively, for control (TABLE 4) Such ratios observed in tissue rapidly synthesizing glycogen are 75 to 100 times removed from phosphorylase equilibrium in the direction of glycogen degradation

Either phosphorylase was catalyzing the synthesis of glycogen under unusual local conditions at the synthesis site, or the synthesis was being catalyzed by another set of reactions independent of inorganic phosphate, an alternate mechanism Another reason for considering the latter possibility was brought out by the work of Sutherland,[18] who has shown that activation of phosphorylase in either liver or muscle by epinephrine or glucagon is associated with glycogen breakdown.

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These workers reported the direct polymeriza tion of UDPG to glycogen without the intervention of glucose-1-phosphate as intermediate by an enzyme present in liver

tous, since they were done under different conditions experiments were done with concentrations of UDPG of 20 μm/ml, with net conversions of the order of 20 per cent of the UDPG to glycogen in a 60-min. incubation period. Recently we have found that the supernatant fluid also contains the enzyme, so that these estimates of activity are ... bly somewhat low.

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observed in these experiments This enzyme has recently been obtained in soluble form and partially purified. It is of interest in terms of possible control mechanisms that, in confirmation of unpublished findings of Leloir and

his co-workers, the glycogen-synthesizing enzyme shows an almost absolute dependence on small amounts of added glucose-6-phosphate (TABLE 6). The amounts required for maximal activation are of the range of hexose-6-phosphate that are present in diaphragm.

UDPG pyrophosphorylase has been measured spectrophotometrically by the pyrophosphate-dependent formation of glucose-1-phosphate. The enzyme is present in the soluble fraction of muscle extracts obtained after centrifugation at 100,000 g for 60 min. Its activity is about ten- to twentyfold higher than the glycogen transglucosylase (TABLE 7) and has a widespread distribution in animal tissues. The combination of these two activities is a reaction

TABLE 5

UDPG GLYCOGEN TRANSGLUCOSYLASE ACTIVITY OF RAT MUSCLE EXTRACTS

	Chemical* mg/100 gm/hr	Spectrophotometric† mg/100 gm/hr
	576	610
	486	783
	210	334
	986	465
	240	527
Mean	500 (2 78 mmoles)	543 (3 02 mmoles)

(Worthington), enzyme, 0 01 ml particles, volume, 1 ml

TABLE 6

EFFECT OF GLUCOSE 6 PHOSPHATE ON UDPG GLYCOGEN TRANSGLUCOSYLASE ACTIVITY

	γ/Mg protein/hour
No glucose 6-phosphate added	4 05
5×10^{-4} M glucose-6-phosphate	91 30

sequence leading to glycogen synthesis from glucose-1-phosphate independently of inorganic phosphate

Discussion

The presence of these two enzymes opens the question of the enzymatic mechanism of glycogen synthesis and degradation in muscle and other tissues. At present there appear to be two reasons for considering a cyclic mechanism involving the uridine-linked pathway for synthesis and phosphorylase for degradation (1) the experiments on phosphorylase activation associated with glycogen breakdown and (2) the present experiments indicating that glycogen synthesis occurs at ratios of inorganic phosphate to glucose-1-phosphate highly unfavorable to synthesis via the phosphorylase system

The physiological significance of this over-all system may be considered in terms of the two following mechanisms (1) a system lending itself much more readily to control mechanisms and (2) a pH-dependent system in which glycogen synthesis would be highly favored thermodynamically at neutral pH Another question raised by the present experiments is the following. Can t' increased glycogen synthesis observed in these experiments in the presence of

to be increased at a medium glucose concentration of 280 mg. per 100 ml under conditions where glucose uptakes were elevated, and the fact that there

Table 7
UDPG Pyrophosphorylase Activity of Rat Muscle Extracts

	Spectrophotometric activity* mg /100 gm /hr
	8430
	7800
	8820
	10000
	8110
	10650
	8600
	8150
	7670
	8420
Mean	8665 (48 1 mmoles)

an elevated hexo

together constitu evidence for the fact that the glycogen synthesis observed insulin does not appear to alone In view of the expe of the activation of the UDPG glycogen transglucosylase by added glucose-phosphate, two factors may be considered Further experiments will required to assess the significance of these factors in the relationship of glyco gen synthesis to the transport action of insulin in muscle

Summary

Balance experiments carried out with rat diaphragms after short-term e periments at 2 concentrations of medium glucose indicate that more than per cent of the increased glucose disappearing from the medium in the pre of insulin can be accounted for as tissue glycogen. The increase in gl

occurs in a perchloric acid-soluble fraction, with a smaller increase in the residual fraction at the higher medium glucose level

Inorganic phosphate, phosphocreatine, *ADP*, and *ATP* are unchanged under these conditions, which is compatible with an insulin-stimulated turnover of high-energy phosphate compounds Under our conditions hexose-6-phosphate [illegible] lin-stimu- [illegible] at ratios [illegible]

Enzyme activities measured in muscle extracts indicate the presence of UDPG pyrophosphorylase and UDPG transglucosylase in sufficient activity to account [illegible] cyclic mech[illegible] dence is pre[illegible] does not appear to be explained solely by the increased penetration of glucose

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The physiological significance of this over-all system may be considered in terms of the two following mechanisms (1) a system lending itself much more readily to control mechanisms and (2) a pH-dependent system in which glycogen synthesis would be highly favored thermodynamically at neutral pH. Another question raised by the present experiments is the following: Can the increased glycogen synthesis observed in these experiments in the presence of

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	8430
	7800
	8820
	10000
	8110
	10650
	8600
	8150
	7670
	8420
Mean	8665 (48 1 mmoles)

is (3) no elevation of the glucose-1-phosphate fraction with an elevated hexose-6-phosphate in the presence of insulin

While none of these alone is conclusive, all three taken together constitute evidence for the fact that the glycogen synthesis observed in the presence of insulin does not appear to be explained by the increased penetration of glucose alone. In view of the experiments of Shaw and Stadie[19] and the demonstration of the activation of the UDPG glycogen transglucosylase by added glucose-6-phosphate, two factors may be considered Further experiments will be required to assess the significance of these factors in the relationship of glycogen synthesis to the transport action of insulin in muscle

Summary

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of insulin can be accounted for as tissue glycogen. The increase in glycogen

as measured with raffinose, sucrose, or thiosulfate, denoted intracellular accumulation, whereas an equal or smaller value indicated that glucose was confined to the extracellular water. The intracellular concentration of glucose is an index of the relative rates of glucose penetration and phosphorylation by hexokinase. An increase in the intracellular concentration of the free sugar can result either from a relative increase in the rate of penetration or from a relative decrease in the rate of phosphorylation. In either circumstance, the presence of glucose within the cell would indicate that phosphorylation was rate limiting. On the other hand, the absence of free sugar from the intracellular compartment would mean that the transport of glucose across the cell membrane was the rate-limiting step in glucose utilization.

The second method consisted of measuring the rate of uptake of the glucose analogue 2-deoxyglucose by two different *in vitro* rat diaphragm preparations: (1) the usual isolated rat hemidiaphragm described by Gemmill[10] and referred to in this paper as the *cut* preparation, and (2) the *intact* diaphragm preparation developed in our laboratory.[5] A more detailed discussion of the physiological and metabolic properties of these preparations will be given in a subsequent section. The 2-deoxyglucose is phosphorylated by hexokinase, $K_m = 2.4 \times 10^{-4}M$,[11] to form the phosphate ester 2-deoxyglucose-6-phosphate, which accumulates within the cell, since it is not acted upon at an appreciable rate by other muscle enzymes. Methods have been developed for the determination of free and phosphorylated 2-deoxyglucose in the presence of each other,[4] thereby permitting the investigation of intracellular penetration and phosphorylation as separate processes.

Effect of Insulin and Epinephrine on the Glucose Distribution in the Diaphragm and Gastrocnemius of Nonfasted Rats

Glucose was confined to the extracellular space in both the gastrocnemius and diaphragm of the nonfasted rats (TABLE 1, columns 1 and 7) at plasma glucose levels ranging from 98 to 163 mg per cent. Intracellular accumulation did occur, however, following the injection of epinephrine (TABLE 1, columns 2 and 8). This was not a result of hyperglycemia per se, for glucose remained extracellular when correspondingly high plasma levels were attained by the intravenous administration of a glucose load (TABLE 1, column 3). Insulin, on the other hand, had a variable effect, depending on the degree of hypoglycemia induced. With moderate hypoglycemia, the percentage of distribution of glucose in the extracellular water was decreased (TABLE 1, column 4). With severe hypoglycemia (TABLE 1, column 5), which results in an endogenous discharge of epinephrine, glucose accumulated within the muscle cell. A concomitant effect of epinephrine secretion is the intracellular accumulation of glucose-6-phosphate,[12, 13] a noncompetitive inhibitor of hexokinase ($K_i = 4 \times 10^{-4} M$)[14] arising from an accelerated rate of glycogenolysis. It seems probable that the accumulation of intracellular glucose occurring during epinephrine secretion resulted from an inhibition of phosphorylation secondary to the elevated phosphate ester concentrations ($> 2 \times 10^{-3}M$) observed under these conditions. The intracellular accumulation of glucose associated with severe insulin hypoglycemia could then be explained as reflecting an accelerated rate of glucose entry (insulin effect) and a decreased rate of phosphorylation (epi-

REGULATION OF GLUCOSE UPTAKE BY MUSCLE: FUNCTIONAL SIGNIFICANCE OF PERMEABILITY AND PHOSPHORYLATING ACTIVITY*

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Much information concerning the pathways and individual enzymes involved in carbohydrate metabolism has been obtained from studies with cell-free systems Similar studies with intact cells or multicellular systems, however, have frequently yielded results that appear to be at variance with this information It is because of these discrepancies that there is an increasing awareness of the importance of structural elements for biochemical events in the intact cell What, for example, are the functional limitations imposed by cell structure on enzymatic processes? Progress has been slow in this area primarily because of the lack of adequate techniques for measuring discrete reactions within intact cells The ultimate goal of such an approach is, of course, an understanding of the processes that determine the integration of

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of poorly [illegible] transfer of sugars across the muscle cell membrane and the effect thereon of various hormones, dietary regimens, and muscular activity.[6-9] The fact, however that these sugars are poorly metabolized limits their usefulness in clarifying

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* The work reported in this paper was supported in part by Grant A-1921 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md

† John and Mary R Markle Scholar in Medical Science.

as measured with raffinose, sucrose, or thiosulfate, denoted intracellular accumulation, whereas an equal or smaller value indicated that glucose was confined to the extracellular water The intracellular concentration of glucose is an index of the relative rates of glucose penetration and phosphorylation by hexokinase. *An increase in the intracellular concentration of the free sugar can result either from a relative increase in the rate of penetration or from a relative decrease in the rate of phosphorylation* In either circumstance, the presence of glucose within the cell would indicate that phosphorylation was rate limiting On the other hand, the absence of free sugar from the intracellular compartment would mean that the transport of glucose across the cell membrane was the rate-limiting step in glucose utilization

The second method consisted of measuring the rate of uptake of the glucose analogue 2-deoxyglucose by two different *in vitro* rat diaphragm preparations (1) the usual isolated rat hemidiaphragm described by Gemmill[10] and referred to in this paper as the *cut* preparation, and (2) the *intact* diaphragm preparation developed in our laboratory[2] A more detailed discussion of the physiological and metabolic properties of these preparations will be given in a subsequent section The 2-deoxyglucose is phosphorylated by hexokinase, K_m ~ 2.4×10^{-4}M,[11] to form the phosphate ester 2-deoxyglucose-6-phosphate, which accumulates within the cell, since it is not acted upon at an appreciable rate by other muscle enzymes Methods have been developed for the determination of free and phosphorylated 2-deoxyglucose in the presence of each other,[4] thereby permitting the investigation of intracellular penetration and phosphorylation as separate processes

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nephrine effect). Consonant with this interpretation is the finding that insulin administered simultaneously with epinephrine did not counteract the effect of the latter on glucose distribution, but increased the intracellular accumulation of free sugar (TABLE 1, columns 6 and 10).

Variations in the endogenous secretion of epinephrine have been avoided by adrenalectomizing animals 30 to 60 min. before the experimental period, this procedure, in essence, depletes the animal of epinephrine without producing adrenocortical insufficiency. In these rats insulin had only one effect even

TABLE 1*

INFLUENCE OF EPINEPHRINE AND INSULIN ON GLUCOSE DISTRIBUTION IN THE DIAPHRAGM AND GASTROCNEMIUS OF THE NONFASTED RAT**

Column No	Epinephrine†	Insulin††	Diaphragm			Gastrocnemius		
			C_p (mg./100 cc.)	C_m (mg./100 cc.)	$C_m/C_p \times 100$ (percentages)	C_p (mg./100 cc.)	C_m (mg./100 cc.)	$C_m/C_p \times 100$ (per cent ages)
Intact animal								
1	−	−	130 (6)	35 4	27 2	163 (10)	25 1	15 4
2	+	−	354 (3)	115 2	32 5	190 (4)	67.4	35 7
3‡	−	−	262 (3)	72 4	27 6	288 (5)	43 0	14 9
4	−	+	66 (5)	13 6	20 6	64 (5)	7.9	12 3
5	−	+	—	—	—	53 (5)	13.8	26 2
6	+	+	248 (3)	133 9	54 0	—	—	—
Acute adrenalectomy§								
7	−	−	98 (4)	20 4	20 8	112 (3)	13.2	11 8
8	+	−	372 (2)	129 5	34 8	155 (1)	51.0	32 8
9	−	+	61 (4)	6 2	10 2	40 (3)	3.1	7 9
10	+	+	264 (3)	131 5	49 8	—	—	—

when hypoglycemia was severe, namely, a marked depletion of glucose in the extracellular space of muscle (TABLE 1, column 9) This depletion suggests that, under conditions of rapid uptake and low plasma glucose levels, the diffusion of sugar in the interstitial fluid from the capillaries to the cell surface may become rate limiting. Of further interest is the finding that intracellular glucose did not accumulate in acutely adrenalectomized rats given insulin and large glucose

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tion, providing epinephrine secretion is prevented.

Effect of Insulin and Epinephrine on the Glucose Distribution in the Intact Diaphragm Preparation of Nonfasted Rats

Glucose could not be demonstrated inside the muscle cell of the *intact* diaphragm either in the presence or absence of added insulin (TABLE 2) Because of the remarkable similarity between the glucose distribution in the diaphragm of the acutely adrenalectomized rat and that in the *intact* preparation in the presence and absence of insulin (TABLE 2), it occurred to us that the *in vitro* [illegible]

Recent studies with the *intact* preparation have shown that the increase in glucose-6-phosphate concentration following epinephrine addition exactly paral-

TABLE 2
INFLUENCE OF EPINEPHRINE AND INSULIN ON GLUCOSE DISTRIBUTION IN THE *IN VITRO INTACT* DIAPHRAGM PREPARATION*

Temperature (centigrade)	Distribution of glucose in tissue water	
	C (percentages)	*I* (percentages)
37°	18 1 ± 0 9 (20 8)	11 1 ± 1 2 (10 2)
27°	21 7 ± 1 6	15 9 ± 0 8
27°†	37 2 ± 2 6 (34 8)	49 6 ± 3 5 (49 8)
17°	29 2 ± 1 3	23 3 ± 1 1

[illegible] *C* [illegible] am) [illegible] are [illegible] ncu- [illegible] for analysis.

leled the accumulation of intracellular glucose, and was of sufficient magnitude to account for at least a 70 per cent inhibition of hexokinase Additional evidence indicating that epinephrine is capable of causing an inhibition of phosphorylation is presented in TABLE 3 In these experiments, the rates of 2-deoxyglucose penetration and phosphorylation were determined in the presence of insulin and epinephrine Although phosphorylation was inhibited about 50 per cent, sugar entry was not affected

Influence of Fasting and Glucose Loading on Glucose Distribution in Rat Diaphragm In Vivo

Glucose was confined to the extracellular space of the diaphragm in rats fasted up to 72 hours (TABLE 4) More prolonged periods of starvation, however, were associated with the appearance of free glucose in the muscle cell of both the diaphragm and gastrocnemius In order to avoid variations in the rate of endogenous epinephrine secretion, which is known to be influenced by

starvation,[13] the remainder of the experiments recorded in TABLE 4 were performed in acutely adrenalectomized rats

Although insulin produced a depletion of extracellular glucose in the 24-hour-fasted adrenalectomized rat identical to that observed in the nonfasted control (TABLES 1 and 4), it was still possible to demonstrate that this short period of fasting produced a marked metabolic change in muscle. Plasma glucose levels averaging 389 mg per cent were not associated with the intracellular accumula-

TABLE 3*

EFFECT OF EPINEPHRINE ON THE PENETRATION AND PHOSPHORYLATION OF 2-DEOXYGLUCOSE†

Period of incubation (min)	2 Deoxyglucose 6-phosphate (μmoles/cc)	2 Deoxyglucose (μmoles/cc)	Total uptake (μmoles/cc)
30	19 9	10 7	30,6 (33 6)
45	25 1	15 4	40,5 (41 5)

* Reproduced from Kipnis and Cori[4] by permission from *The Journal of Biological Chemistry*

† Diaphragms were incubated in 0 06 M 2 deoxyglucose with insulin (0 4 units per cc) added to increase the rate of penetration The concentration of epinephrine was 6×10^{-4} M The values are averages of 3 experiments The values in parentheses represent the uptake of 2-deoxyglucose in the absence of epinephrine

TABLE 4*

INFLUENCE OF FASTING AND OF GLUCOSE LOAD ON DISTRIBUTION OF GLUCOSE IN RAT DIAPHRAGM**

Length of fasting period (hours)	Insulin†	Glucose load‡	C_p (mg /100 cc)	C_m (mg /100 cc)	$C_m/C_p \times 100$ (percentages)
Intact animal					
24			122 (6)	32 8	26 9
72			108 (3)	25 5	26 4
120			114 (3)	34 1	29 8
Adrenalectomized animal§					
24			116 (3)	23 8	20 6
24	+		66 (3)	4 4	6 7
0		+	400 (4)	89 9	22 5
0	+	+	389 (4)	108 0	27 8
24		+	380 (3)	102 3	26 9
24	+	+	500 (3)	311 0	60 2
72		+	225 (3)	77 5	34 2
72	+	+	239 (3)	112 8	47 2
96		+	342 (3)	177 5	51 8
96	+	+	328 (3)	230 8	70 4

* [illegible] from Kipnis *et al.*[13] by permission from *The Journal* [illegible] *Chemistry*

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tion of glucose in nonfasted rats even after insulin administration. In 24-hour-fasted animals, however, similar conditions resulted in the accumulation of large quantities of glucose within the muscle cell Furthermore, in animals fasted for from 72 to 96 hours, injection of glucose alone produced this effect, which was reinforced by the simultaneous administration of insulin

It is known that prolonged starvation results in an impaired glucose tolerance.[15, 16] Obviously, this impairment is not a result of epinephrine secretion, *nor is it secondary to an impairment of glucose entry*, since intracellular glucose accumulated in the fasted animal It would appear from these findings that the phosphorylating capacity of muscle is severely impaired by starvation

TABLE 5*

GLUCOSE DISTRIBUTION IN DIAPHRAGMS OF ALLOXAN-DIABETIC RATS**

Length of fasting period (hours)	Other experimental procedures	C_p (mg /100 cc)	C_m (mg /100 cc)	$C_m/C_p \times 100$ (percentages)
0	—	686 (4)	191	27 8
0	*Insulin*†	750 (3)	321	42 8
24	—	568 (5)	154	27 1
24	Insulin	340 (4)	158	46 5
24	Adrenalectomy‡	510 (3)	144	28 4
0	Adrenalectomy‡ + insulin†	315 (3)	138	43 8
0	Epinephrine§	650 (2)	303	46 6

* Reproduced from Kipnis *et al*[13] by permission from *The Journal of Biological*

Preliminary studies suggest that this effect is mediated via pituitary and adrenal cortical secretions

Influence of Alloxan Diabetes on Glucose Distribution in Diaphragm In Vitro

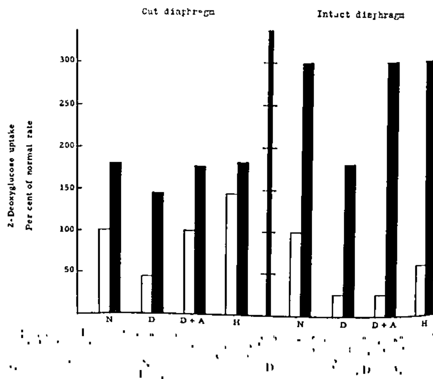
The effects of fasting have often been compared to the diabetic state and, as

fore, that the phosphorylating capacity of diabetic muscle is diminished Whether this is a primary effect of insulin deficiency or is secondary to other

factors is discussed in a later section The effect of epinephrine injection on intracellular distribution is the same in the diabetic as in the normal animal

Uptake of 2-Deoxyglucose by the Cut- and Intact-Diaphragm Preparations

For purposes of presentation, the relative rates of 2-deoxyglucose uptake with and without insulin in the *cut* and *intact* preparations from normal (nonfasted), diabetic, diabetic-adrenalectomized, and hypophysectomized (fasted) rats are recorded in FIGURE 1 The uptake of 2-deoxyglucose by each group



has been compared on a percentage basis with the diaphragm of the normal nonfasted animal without added insulin The results with the *cut* preparation ai ... stigators[17-20] T ... xan diabetic r ... . Although insulin stimulated utilization in this preparation, the final rate attained is significantly lower than that of normal insulinized muscle. The *cut* diaphragm of the diabetic-adrenalectomized animal (adrenalectomy was performed 4 to 7 days after the diabetes was established and 4 days before the animal was sacrificed) was indistinguishable from that of the normal rat either in the presence or absence of insulin. Hypophysectomy resulted in a significant increase in

the basal rate of utilization and a proportionately decreased stimulation by insulin.

The results obtained with the *intact* diaphragm differ markedly from those of the *cut* preparation. The stimulatory effect of insulin on 2-deoxyglucose uptake is greater in the *intact* than in the *cut* preparation. Furthermore, the *intact* diaphragm of the alloxan-diabetic rat exhibited a far greater impairment of uptake than is observed in the *cut* preparation. Insulin failed, as it did with the *cut* preparation, to restore the rate of uptake of the diabetic *intact* diaphragm to the level observed in normal insulinized muscle. The most striking differences were seen with the diabetic-adrenalectomized and hypophysectomized (fasted) animals. Contrary to the findings with the *cut* preparation, adrenalectomy, in the absence of insulin, did not alter the severely impaired utilization characteristic of the *intact* diaphragm of diabetic animals. In the presence of insulin, however, the rates of utilization were similar to those of

d, in the *intact*

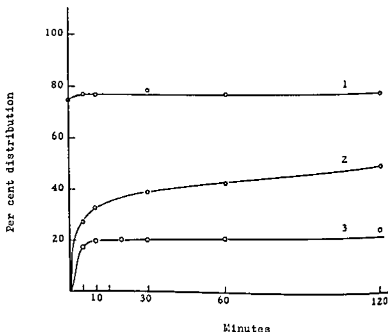
2-deoxyglucose

Before discussing the last group of experiments, it is first necessary to examine the physiological properties of the *cut* and *intact* preparations and consider their metabolic implications. The usual isolated rat hemidiaphragm[10] has been referred to in this paper as the *cut* preparation because all of its muscle fibers are *cut* at both ends during its excision from its attachments to the rib cage, spine, sternum, and central tendon. The *cut* diaphragm has been used to study a variety of metabolic problems of muscle that involve the penetration as well as the utilization of sugars. A series of studies designed to determine whether the *cut* diaphragm is a suitable *in vitro* test object for permeability measurements led to the conclusion that it is inadequate for this purpose.[3, 11] For example, a variety of substances that are normally excluded from the cell interior, such as sucrose, raffinose, thiosulfate (FIGURE 2), inulin, and ferricyanide, readily penetrate into the intracellular compartment of the *cut* diaphragm. These shortcomings are not associated with the *intact* diaphragm, since none of the muscle fibers are damaged in its preparation. This preparation is similar to the diaphragm in the living animal with respect to total tissue water, intracellular and extracellular spaces (FIGURE 2), insulin responsiveness, and rates of sugar penetration.[3]

We have recently studied, in collaboration with Dr. Adolph Cohen, the electron microscopic appearance of the *intact* and *cut* preparations following prolonged incubation in Krebs-Henseleit phosphate buffer. The *intact* preparation could not be distinguished from the diaphragm removed from the living animal and immediately fixed for study; the *cut* preparation, on the other hand, exhibited severe and progressive cellular deterioration. The mitochondria were swollen and contained fragmented cristae, the muscle fibrils were thickened and indistinct, and the endoplasmic reticulum was markedly engorged.

The rates of intracellular penetration of both metabolizable and nonmetabolizable sugars are much faster in the *cut* preparation than in the *intact* one. For example, the cellular entry of 2-deoxyglucose is fourfold and that of D-xylose is tenfold more rapid in the *cut* than in the *intact* diaphragm. Not

only are the permeability characteristics to sugars affected, but Menozzi *et al*[22] have recently shown that ion transport is also markedly altered. Serious doubt, therefore, may be entertained as to the functional significance of the cell membrane as a metabolic regulator in the *cut* preparation. For example, no difference in permeability could be observed between the normal and the diabetic *cut* preparations using the nonmetabolizable insulin-responsive pentose D-xylose, even though the utilization of glucose and 2-deoxyglucose was decreased 50 per cent (FIGURE 3) In view of these findings, it appears likely that the utilization rates observed with the *cut* preparation are representative of



the phosphorylating capacity of muscle, whereas those obtained with the *intact* preparation are a measure of the rate of intracellular transport.

The results that have been presented indicate that other factors, in addition to the permeability properties of the cell membrane, may limit the rate of glucose metabolism in muscle Since the first intracellular event in glucose metabolism is its phosphorylation by hexokinase, it is reasonable to expect that this reaction may function as one of the regulators of glucose utilization The fact that free glucose can be demonstrated within the cell in a variety of conditions supports this contention For example, epinephrine, whether introduced by injection or discharged from the adrenals in response to hypoglycemia or other stimuli, such as muscle work, leads to an intracellular accumulation of free sugar. As mentioned previously, this reaction probably results from a decrease in the

rate of phosphorylation caused by the associated elevation in the glucose-6-phosphate concentration The decreased glucose tolerance associated with fasting is, in part, a reflection of a diminished rate of phosphorylation in muscle, since free sugar accumulates intracellularly under conditions in which it would have remained extracellular in the nonfasted animal The muscle of the diabetic animal, in addition to a defective sugar transport system, is also characterized by an impaired phosphorylating capacity Thus, free sugar accumulates within the cell, both *in vivo* and *in vitro*, upon the addition of insulin It would appear, however, that membrane permeability is more seriously affected by insulin deficiency than is the phosphorylating capacity, since the rate of 2-deoxyglucose utilization by the diabetic *intact* diaphragm preparation is 10 to 20 per cent of normal as compared to a 50 per cent decrease in the diabetic *cut* diaphragm This is in agreement with the results of recent experiments by Field and Cori[28] in which the rates of glucose utilization in nor-

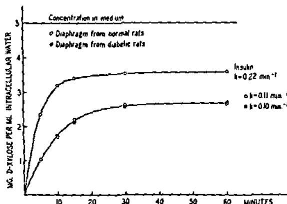


FIGURE 3 The rate of D-xylose penetration in the *cut* diaphragm of normal and alloxan diabetic rats.

phosphorylation, either *in vivo* or *in vitro*, suggests that this defect is a secondary effect of insulin deficiency.

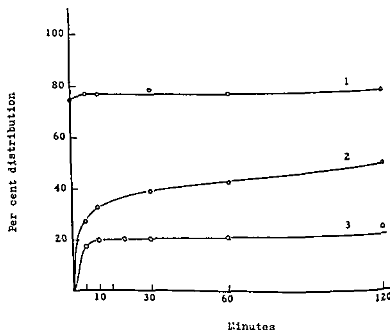
It is possible to reconcile the divergent results obtained with the *cut* and *intact* preparations of the diabetic-adrenalectomized and hypophysectomized

as 2-deoxyglucose and nonmetabolizable sugars such as D-xylose. One would not expect to observe any effect of the injection or removal of adrenal or pituitary secretions on glucose uptake under conditions where penetration was rate

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limiting This observation is consistent with the results of Long and Lukens[24] and of others[25-27] who have shown that the carbohydrate tolerance of depancreatized animals is not improved by the removal of the adrenals It is also in agreement with clinical observations that diabetes can occur in individuals with adrenal cortical insufficiency, and that the impaired glucose tolerance of the diabetic human is not corrected by adrenalectomy. Under conditions where penetration is not rate limiting, however, the effects of adrenal cortical ablation become evident This is the situation in the diabetic *cut* diaphragm either with or without added insulin and in the diabetic *intact* preparation with added insulin These results indicate that the impaired phosphorylating capacity of diabetic muscle is, at least in part, a reflection of adrenal cortical activity.

Houssay and his associates, in their classic studies on the effects of hypophysectomy on carbohydrate metabolism,[28-30] demonstrated that the hyperglycemic response to a glucose load was prolonged, that the extrahepatic consumption of sugar was decreased, and that the sensitivity to insulin was markedly increased These original observations have since been confirmed in the living animal by numerous investigators using a variety of experimental techniques.[31-35] Similar results have now been obtained with the *intact* diaphragm Since penetration is rate limiting in this *in vitro* preparation, the impaired uptake of sugar can be attributed to a diminished rate of transfer across the cell membrane reflecting, I believe, the decreased levels of insulin activity in the hypophysectomized animal[36] These findings, however, are in contrast to the increased rate of utilization and the decreased insulin response observed in the *cut* diaphragm of the hypophysectomized animal. These results would be anticipated if, as previously proposed, the pituitary secretions depress phosphorylating activity, since utilization rates in the *cut* diaphragm reflect its phosphorylating capacity.

The intracellular [illegible]

[illegible] utilization. The removal of this restraining influence would then be one of the factors contributing to the increased insulin sensitivity of the adrenalectomized or hypophysectomized animal.

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ROLE OF INSULIN IN TWO PATHWAYS OF GLUCOSE METABOLISM *IN VIVO* GLUCOSAMINE AND GLYCOGEN SYNTHESIS*

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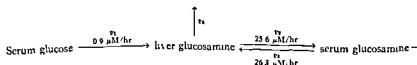
One manner of further localizing the action of insulin is to compare its in the regulation of two pathways of glucose metabolism.

For this purpose, a comparison was made of the biosynthesis of liver gl gen and protein-bound glucosamine in the insulin-deficient state. This done by measuring the incorporation of radioactivity from C^{14}-labeled glu into the liver glucosamine and glycogen of the intact alloxan diabetic rat comparing these values to those previously reported for the normal rat.[1]

It was of interest to compare the synthesis of liver glycogen to tha glucosamine in particular because an enzyme has been described in liver synthesizes glucosamine from glucose-6-phosphate,[2] and also because it been shown in a previous study that the liver is the principal site of syntl

pathological states [3]

A study of the biosynthesis of glucosamine from C^{14}-labeled glucose in normal intact rat indicated a very rapid turnover of this substance, wi turnover time of 0 8 hour for the liver glucosamine and 2.0 hours for the se glucosamine The following scheme was proposed to show the relations between the serum glucose and the liver and serum glucosamine.[1]



From the rates indicated for a 250-gm rat it may be seen that, in additio the synthesis of new glucosamine molecules from glucose, a very rapid change takes place between the glucosamine of liver and serum

The data given in the present study show that in diabetic rats the bio thesis of glucosamine from glucose is essentially unimpaired, despite an alr complete inability of these animals to synthesize liver glycogen from gluc

Experimental

Male albino rats of the Wistar strain were used All animals were fed Purina during the experiment to the time of sacri intravenous injection of alloxan monohydrate (37 to 39 mg./kg) after a

* The work reported in this paper was supported in part by a Postdoctoral Fellow from the American Cancer Society, Inc, New York, N Y.

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hour fast The animals were used for the experiments between 1 and 4 months after alloxan injection, and then only if their blood glucose was greater than 300 mg per cent. Each animal was given a single intraperitoneal injection of 10 to 15 μc glucose-U-C^{14} (2.08 μc/mg) in 2 ml of physiological saline. At various time intervals following the injection of the radioactive glucose, 0.2 ml of blood was taken from the tail vein of each rat without anesthesia for the purpose of determining the blood glucose specific activity The animals were sacrificed at various times following the injection of the glucose-C^{14} by exsanguination by way of the inferior vena cava under light ether anesthesia

The details of the isolation procedures, as well as of the chemical and isotopic analyses, were identical to those previously described for normal animals[1] The protein-bound glucosamine was isolated from the trichloracetic acid precipitates of liver, kidneys, lungs, testes, and spleen, and from the ethanol precipitate of serum The glucosamine was liberated from the protein precipitates by acid hydrolysis and then separated from neutral sugars on a Dowex-50 cation exchange column The amino sugar was then converted to the glucose phenylosazone for the purpose of determining its specific activity The liver and kidney glycogen was precipitated from the trichloracetic acid extracts by the addition of ethanol and, following acid hydrolysis, the glucose phenylosazone was prepared for counting its radioactivity Likewise, the activity of blood and urine glucose was determined from the glucose phenylosazone formed from the Somogyi filtrates

Colorimetric and chromatographic analyses of the Dowex-50 eluates of hydrolyzed diabetic tissues revealed the same very high ratio of glucosamine to galactosamine that is found in normal tissues[1]

All measurements of radioactivity were adjusted to 5.0×10^6 cpm injected into a 250-gm rat

Results

Incorporation of radioactivity into glucosamine The specific activity and total activity of the protein-bound glucosamine of liver and serum following the injection of a tracer dose of glucose-C^{14} are shown in TABLE 1 From the ratio of the specific activity of the liver glucosamine to that of the serum glucosamine it may be seen that, while the liver is more active than the serum early in the course of the experiment, the serum activity soon becomes equal to and then exceeds that of the liver (TABLE 1) The activity in the protein-bound glucosamine of several other tissues was also determined at 3.75 hours following the injection of the glucose-C^{14} (TABLE 2)

Incorporation of radioactivity into liver and kidney glycogen Very little activity was incorporated into the liver glycogen from glucose in any of the animals studied (TABLE 1) Very high levels of kidney glycogen were present (TABLE 2), in contrast to normal animals, which were found to have only about

imals

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incorporation of radioactivity into the glucosamine of normal and diabetic rats, it was necessary to take into account any differences in the specific ac-

tivity of the blood glucose in these two types of animals. For this purpose, several samples of blood were obtained from each rat following the injection of glucose-C^{14}, the specific activities at each time are plotted in FIGURE 1

TABLE 1

RADIOACTIVITY IN LIVER GLUCOSAMINE, SERUM GLUCOSAMINE, AND LIVER GLYCOGEN FOLLOWING GLUCOSE C^{14} INJECTION INTO DIABETIC RATS

Rat No	Time Hours	Liver glucosamine			Serum glucosamine		Liver-specific activity / Serum-specific activity	Liver glycogen	
		Specific activity cpm/μmole	Quantity* μmoles	Total activity cpm	Specific activity cpm/μmole	Total† activity cpm		Quantity* μmoles glucose equivalents	Total activity cpm
22	1 50	8 6	28 5	246	5 0	229	1 74	251	70 6
6	3 75	17 6	27 9	491	17 9	810	0 98	354	66 2
7	3 75	14 7	26 5	388	14 5	667	1 01	264	50 4
20	3 75	11 1	26 7	297	11 6	534	0 96	73	15 6
26	12 00	7 2	25 0	179	9 6	444	0.74	88	16 7
8‡	3 75	50 8	25 6	1312	49 3	2270	1 03	500	505 0

fro
body weight
‡ Fructose C^{14} injected instead of glucose C^{14} under the same conditions

TABLE 2

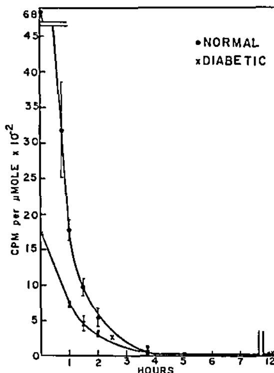
RADIOACTIVITY IN GLUCOSAMINE OF SEVERAL TISSUES AND IN KIDNEY GLYCOGEN FOLLOWING GLUCOSE-C^{14} INJECTION INTO DIABETIC RATS

Rat No	Kidney				Lung		Testes		Spleen	
	Glycogen		Glucosamine							
	Quantity μmoles glucose equivalents	Total activity cpm	Specific activity cpm/μmole	Total activity cpm	Specific activity cpm/μmole	Total activity cpm	Specific activity cpm/μmole	Total activity cpm	Specific activity cpm/μmole	Total activity cpm
6	66 6	83	8 34	117	30 1	293	5 13	19 1	14 1	18 7
7	75 5	116	6 26	86	11 0	83	6 35	21 7	8 1	15 5
20	197 0	156	5 53	75						

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250 gm

The diabetic animals had an average blood glucose level of 561 mg per cent compared with 116 mg. per cent for the normal animals, and excreted an average of 142 mg. of glucose in the urine per hour per 100 gm of body weight, causing a loss of 58 per cent of the injected radioactivity during the course of the experiment The body glucose pools, calculated by the method of

animals is considerably greater than that of the diabetic animals In order to compare the activity in the glucosamine and glycogen in the diabetic animals with the activity of these substances in the normal animals, it is first



ratio of the area under the normal specific-activity curve to that under the diabetic specific-activity curve, the following correction factors were calculated 3 83 for 1 5 hours, 3 18 for 3 75 hours, and 3 11 for 12 hours following the glucose injection *

* Since the equations for the specific activity of a product at any time involve the integral of the specific activity of the precursor up to that time, the necessary correction factors for

As may be seen from TABLE 3, the pool sizes for the liver and serum glucosamine were essentially the same in normal and diabetic animals, whereas the liver glycogen pool was markedly reduced in the diabetic.

In TABLES 4 and 5 the diabetic values for both glucosamine and glycogen corrected to the normal serum glucose-specific activity by multiplication by the above factors are given alongside mean normal values for the same time intervals. At the time intervals shown, there is little difference between the total activity of the glucosamine of normal or diabetic animals either in liver

TABLE 3

COMPARISON OF POOL SIZES IN NORMAL AND DIABETIC RATS

Type of animal	Liver		Serum glucosamine
	Glycogen glucose equivalents	Glucosamine	
Normal ± s.e.	1,720 ± 191	21.5 ± 0.4	50.0 ± 2.0
Diabetic ± s.e.	206 ± 54	26.9 ± 0.6	46.0 ± 5.2

All values expressed as μmoles per 250 gm. rat.

TABLE 4

A COMPARISON OF RADIOACTIVITY IN LIVER GLYCOGEN, LIVER GLUCOSAMINE, AND SERUM GLUCOSAMINE OF NORMAL AND DIABETIC RATS

Time hours	Liver								Total serum glucosamine activity		
	Total glycogen activity			Total glucosamine activity			Glycogen / Glucosamine				
	Normal cpm	Diabetic cpm	Normal / Diabetic	Normal cpm	Diabetic cpm	Normal / Diabetic	Normal	Diabetic	Normal cpm	Diabetic	Normal / Diabetic
1.5	21,138	271	77.9	1157	941	1.23	18.3	0.29	1101	830	1.33
3.75	33,973	140	243.0	1021	1248	0.82	33.2	0.11	2500	2130	1.17
12.0	3,628	52	62.9	531	557	0.96	6.2	0.09	1527	1370	1.11

All counts adjusted to 5.0×10^6 cpm glucose C^{14} injected into a 250-gm. rat.

All values are means for each time, and are expressed per 250 gm. rat, the normal values were reported previously.[1] All activities given for diabetic animals are corrected to the normal serum glucose specific activity as explained in the text.

or in serum. In marked contrast to this essentially normal synthesis of glucosamine from glucose by the diabetic liver, there was a pronounced impairment in the synthesis of liver glycogen from glucose (FIGURE 2). Glycogen activity in the diabetic liver was reduced to about 1 per cent of normal (TABLE

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tal liver glucosamine,

4).

liver glucosamine and glycogen activity were obtained from the geometrical integration of the specific activity of the blood glucose, which is represented by the area under the blood glucose-specific activity curve up to a given time.

As in liver and serum, the other diabetic tissues studied do not appear to show any impairment in the synthesis of glucosamine from glucose (TABLE 5) at the time studied.

TABLE 5

A COMPARISON OF THE RADIOACTIVITY IN THE GLUCOSAMINE OF SEVERAL TISSUES OF NORMAL AND DIABETIC RATS

Tissue	Total Activity		
	Normal cpm	Diabetic* cpm	Normal/Diabetic
Kidney	184†	294	0.63
Lung	250	585	0.43
Testes	70.3	54.4	1.29
Spleen	73.6	64.8	1.13

previously[1]

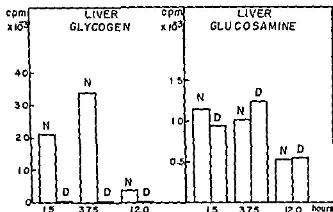


FIGURE 2 A comparison of the total activity in the liver glycogen and glucosamine of normal and diabetic rats The values are means for each time and are expressed per 250 gm rat The activities of the diabetic animals are corrected to the normal serum glucose-specific activity

Incorporation of radioactivity into glucosamine following injection of fructose-U-C^{14} Fructose-U-C^{14} was injected into a diabetic rat under the same conditions as for glucose-U-C^{14}, and the animal was sacrificed 3.75 hours thereafter As observed in normal rats,[1] both liver and serum glucosamine activity increased in the same proportion, so that the ratio of the two remained the same as with glucose (TABLE 1) No significance can be attached to the absolute

increase, since the specific activity of the injected fructose would be d
less than that of the injected glucose

Discussion

It appears from the data of the present study that the synthesis of pr
bound glucosamine from glucose in several tissues of the alloxan diabet

the diabetic animal the liver is the primary site of synthesis of the serum
cosamine, as appears to be the case in the normal animal.[1]

It is of interest that Schiller and Dorfman have observed that the incor
tion of radioactivity from C^{14}-labeled glucose into the hyaluronic acid c
skin of alloxan diabetic rats was reduced to about one third of normal[5]
decreased activity of the hyaluronic acid molecule could represent a decr
synthesis of either the glucosamine or the glucuronic acid moieties, or
Since these investigators did not measure the specific activity of the l
glucose, it is difficult to compare their results with the data obtained i
present study, in which the markedly decreased blood glucose-specific act
of the diabetic animals was taken into account

size glucosamine from glucose at a normal rate despite an almost total im
ment in the synthesis of glycogen suggests that insulin does not play the
role in the regulation of all of the products of glucose metabolism Tha
impairment in glycogen synthesis in the alloxan diabetic rat is due to in
deficiency has been demonstrated in a previous study.[7] In order to att
to explain this difference in the effect of insulin deficiency on the synthes
glycogen and glucosamine, it is necessary to consider the pathway of glu
mine synthesis from glucose Pogell and Gryder have demonstrated the
ence in rat liver of an enzyme that can synthesize glucosamine-6-phosp
from glucose-6-phosphate and glutamine[2] If glucose-6-phosphate is also
precursor of glucosamine in the liver of the intact animal, it would ap
likely that it belongs to a glucose-6-phosphate pool that is not under th
fluence of insulin and is therefore a pool separate from that giving ris
glycogen If the action of insulin on the liver is one affecting the penetra
of glucose, it may be that glucosamine synthesis takes place in a compartm

to insulin In this regard, it is of interest that Shaw and Stadie have d
onstrated that, in the rat diaphragm, glycogen synthesis from glucose is
sponsive to insulin, whereas lactic acid formation from glucose is unaffec
by insulin[8] In order to explain their data, these investigators have po

lated the existence of two separate glucose-6-phosphate pools, one inside the cell that is responsive to insulin, and another on the cell surface

This difference in response to insulin deficiency of the liver glycogen and glucosamine is consistent with the observations made in normal animals, in which there was no correlation between the activity incorporated into the liver glycogen and glucosamine [1]

However, it is also necessary to consider the possibility that there is only one glucose-6-phosphate pool, but that the enzyme responsible for the amination of glucose-6-phosphate has such a high affinity for its substrate that it remains saturated despite a decreased formation of glucose-6-phosphate from glucose, resulting in unimpaired glucosamine synthesis

It is of interest to note that, like the liver, the other tissues reported in this study also synthesize glucosamine from glucose at essentially a normal rate The data of this study may be of some relevance in evaluating the origin of the retinal and glomerular lesions found in human diabetes, which have been postulated to be the result of some disorder of mucopolysaccharide metabolism [9]

Summary

A comparison was made of the biosynthesis from glucose of liver glycogen and protein-bound glucosamine in the intact alloxan diabetic rat From the incorporation of radioactivity from tracer doses of glucose-U-C^{14} into these two substances, it was judged that there was no impairment in the rate of synthesis of the glucosamine despite an almost complete failure in the synthesis of glycogen This difference in response to insulin deficiency of two pathways of glucose metabolism is discussed, and its possible relevance to localizing the site of action of insulin is considered

The synthesis of the protein-bound glucosamine of serum, kidney, lung, testes, and spleen in the diabetic rat was also studied with the aid of glucose-C^{14} and, as in liver, no decrease from the normal was observed

From the relationship of the specific activity of the liver and serum glucosamine at several time intervals, the liver appeared to be the primary site of synthesis of serum glucosamine, as has been reported for the normal rat

Acknowledgment

I express my appreciation to A Baird Hastings for his interest in this work

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Part III. Biochemistry and Physiology of Insulin and the Sulfonylurea Compounds

EFFECT OF 6-DEOXY-6-FLUOROGLUCOSE ON GLUCOSE PERMEATION OF THE CELL*

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San Diego State College, San Diego, Calif.

George S Serif
University of South Dakota, Vermillion, S Dak

The processes involved in the permeation of glucose into muscle cells have received considerable attention in recent years. The possible ways of transfer [illegible] a chemically [illegible] brane carrier [illegible] It is ques[illegible]

[illegible] specificity[1] The use of modified sugars is not always a rewarding one, because one cannot always predict in advance whether or not a particular compound will be useful. Most of the compounds examined thus far have been of no value because of their inertness for one reason or another. However, 6-deoxy-6-fluoroglucose (hereafter referred to as 6-FG) is a compound with interesting physiological properties In this report we present a summary of the effect of 6-FG on the intracellular transfer of glucose.

Preparation of 6-FG and Facts Regarding It

We prepared 6-FG according to the procedure of Helferich *et al*[2,3] as revised by Blakley[4] This compound is a well-defined crystalline compound with the stereochemical configuration of glucose In considering the possibility that this compound may enter into an "enzyme socket," it should be pointed out that the atomic radius of covalent fluorine is smaller than that of the hydroxyl group that it replaces

Glucose Permeation and Inhibitory Action of 6-FG

The physiological properties of 6-FG as previously reported by us are summarized as follows

(1) 6-FG rapidly enters the cells of the extrahepatic tissues and simultaneously inhibits the intracellular transfer of glucose[5]

(2) The rate of cell entry of 6-FG and its final distribution in the tissue water o[illegible] n of insulin, likew[illegible] ased by the presence of [illegible]

* The work reported in this paper was supported in part by Grant A-2949 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md

(3) 6-FG inhibits the oxidation of uniformly labeled glucose-C^{14} in *in vitro* studies using rat kidney slices,[6] rat epididymal adipose tissue, and rat diaphragm tissue[7]

A comparison of the relative effectiveness of 6-FG as an inhibitor of glucose oxidation in rat kidney and adipose and diaphragm tissues is shown in FIGURE

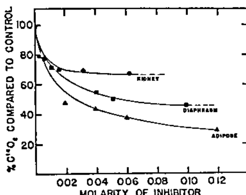


FIGURE 1 Comparison of the effect of 6-FG on the inhibition of glucose (uniformly labeled) oxidation on rat kidney, rat diaphragm, and rat epididymal adipose tissue

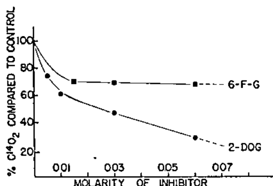


FIGURE 2 Comparison of the effect of 6-FG and 2-deoxyglucose on the inhibition of glucose (uniformly labeled) oxidation in rat kidney slices

1 It is apparent from the data that, as an inhibitor of glucose oxidation, 6-FG is most active in adipose tissue in the series adipose tissue > diaphragm > kidney

The most complete data regarding the action of 6-FG on the entry of glucose into the cells are furnished by the results with kidney tissue FIGURE 2 shows data on the effect of 6-FG concentrations on the conversion of uniformly labeled glucose to CO_2 with this tissue With increasing concentration of 6-FG, the inhibition of radioactive CO_2 formation approaches a plateau,

On the other hand, 2-deoxyglucose maintains an increase in inhibition with increasing concentrations of the inhibitor. With adipose and diaphragm tissue, [illegible] hereas the curves ob- [illegible] bition.[7] With respect [illegible] atively the same with [illegible]

Modes of Cell Permeation as Suggested by These Studies on 6-FG

The plateau type of inhibition curve resulting from these studies is unusual A possible explanation of these data is that 6-FG inhibits a specific pathway of glucose oxidation, but that one or more alternate pathways exist. Under these conditions increasing the concentration of 6-FG ultimately would result in complete inhibition of the susceptible pathway, while the alternate pathway

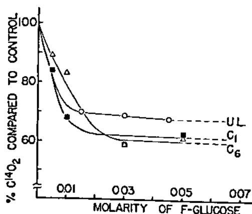


FIGURE 3 Comparison of the effect of 6-FG on the oxidation of C_1, C_4, and uniformly labeled glucose in kidney slices

would be unaffected. From this line of reasoning 2-deoxyglucose would have to inhibit at a point where all glucose oxidation to CO_2 is affected, which is in agreement with the information available for it.[8] If the point of glucose inhibition occurred in the metabolic sequence after the formation of glucose-6-phosphate, then 6-FG inhibition could be tentatively explained in terms of known pathways such as the hexose monophosphate path or the Emden-Meyerhof cycle If either one of these known routes were involved, then one would expect to observe a greatly increased maximum inhibition of CO_2 formation with 6-FG if uniformly labeled glucose were replaced with C_1- or C_4-labeled glucose, depending on which of the two pathways was inhibited. However, since the maximum inhibition of radioactive CO_2 formation from C_1, C_4, and uniformly labeled glucose remains approximately the same, it appears that the inhibition imposed by 6-FG must be effected prior to the formation of glucose-6-phosphate It may be that 6-FG interferes with a process concerned with cell permeation directly. This possibility was suggested by Blakley and Boyer,[9] who examined the effect of 6-FG on yeast fermentation

and concluded from their studies that the compound might be competing with glucose on the cell surface for cell entry. If 6-FG interference occurs at cell entry then, to be consistent with our data, two or more pathways of cell entry must be present. On the other hand, if 6-FG inhibits within the cell, then again, to be consistent with our data, a pathway alternate to that involving hexokinase must be present

If one accepts the explanation for the plateau type of inhibition curve as suggesting that 6-FG inhibits a specific pathway for the oxidation of glucose to CO_2 and that an alternate unaffected pathway also exists, then one must conclude that the sensitive pathway is a common one in kidney slices, in adipose and diaphragm tissue of the rat, and in the extrahepatic tissues of the rabbit, but the extent to which the sensitive pathway operates in each tissue varies.

Summary

We have found that 6-FG inhibits the oxidation of glucose in rat kidney, and in diaphragm and adipose tissue, also that it inhibits the intracellular trans-

same re-
hat 6-FG
ey slices)
when the inhibitor concentration is increased is interpreted to suggest that for glucose two or more pathways for metabolism prior to the formation of glucose-6-phosphate may exist Alternatively, two or more methods of cell entry may exist for glucose

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GLUCOSE TRANSPORT THEORY OF INSULIN ACTION

M. S. Goldstein
Michael Reese Hospital, Chicago, Ill.

About 10 years ago Rachmiel Levine, myself, and a number of colleagues

ing interest in the ever-developing field of enzymology. The search for the site of action of insulin paralleled the development of ideas of enzyme pathways. Each point of confluence of the flow of intermediary steps from protein, carbohydrate, and fat has briefly but temporarily occupied interest as a proposed point of insulin action.

In 1946 Soskin and Levine presented a critical review of this area of work[1]

lar entry of sugar to the action of insulin by a new and independent demonstration. In essence, we were able to dissociate cellular entry of a glucoselike sugar from subsequent enzymatic or metabolic alterations of that sugar.[5]

We have reported experiments with the eviscerated-nephrectomized dog and rat

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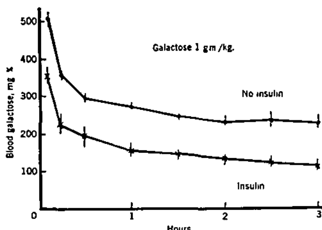
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lation of these materials, being located exclusively in the abdominal viscera, are now absent. Such metabolically inert sugars, when introduced intravenously in the eviscerated-nephrectomized preparation, are distributed in what-

ever water compartments of the body are available to them A family of hexoses and pentoses was found to occupy about 45 per cent of the carcass weight within 60 to 90 min after intravenous administration Beyond this

thereafter, promptly made available to a certain selected group of sugars an additional intracellular compartment, affording distribution of the material in total body water. This is best exemplified by FIGURES 1, 2, 3, and 4, where additional insulin serves to determine, not the rate metabolism of these inert sugars, but rather the area of distribution within the body of specific sugars

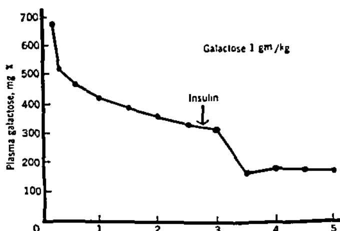


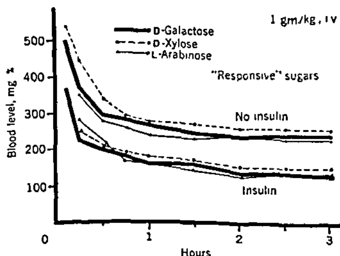
ology[5]

Of the sugars we employed, some proved to be insulin-responsive, while others were unchanged by the presence or absence of insulin Those sugars whose intracellular penetration was promoted by the hormone revealed a common aspect of chemical structure in that they all resembled glucose in the configuration of hydrogens and hydroxyls about carbons 1, 2, and 3 The suggestion was made at that time that a barrier to the entry of sugars existed at the surface of certain cells, presumably skeletal muscle, heart, and adipose tissue This *barrier to entry was overcome by a specific system, located in a* hypothetical cell-membrane structure, that transported glucose from the extracellular to the intracellular position This glucose transport system, while being structurally designed for operation with glucose, would nevertheless accept and transport sugars of related structure sharing a common configuration about the first three carbons Confirmation and extension of both these

findings and their interpretation soon followed, and a direct demonstration for this analogous situation was finally made for glucose[7-12]

Subsequent work has revealed that the specificity of this system is considerably wider in nature than experience with this early group of sugars had revealed. That the hexoses and pentoses possessing the same configurations





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about carbons 1, 2, and 3 as seen in D-glucose makes them acceptable to this insulin-promoted sugar transport system remains unchallenged. Manipulations of the hydrogens and hydroxyls on carbons 2 and 3 indicate that the hydroxyl is not essential and can be replaced by a methyl group, hydrogen, or amine group. At least two sugars in which the configuration about carbon 1 and 2 are different from that of glucose, namely, mannose and fructose, have been reported as active participants in this transport system.[13-17]

These additional characterizations of the specificity of this system have in no way disproved the conception of a uniquely designed apparatus. In the same vein, some demonstrations of token action on other sugars are quite irrelevant.[18] The system appears to behave in a manner that is far from indiscriminate, and insulin can promote increased rates of entry consistent with

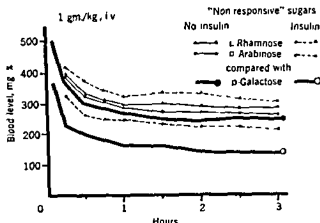


FIGURE 4 The sugar transport action of insulin is quite specific, and not all sugars are affected. The distribution of D-arabinose and L rhamnose in the eviscerated nephrectomized dog is unaffected by added insulin. Reproduced by permission from *The American Journal of Physiology*.[4]

movement of substrate amounts of carbohydrate for only a relatively few structured sugars.

It would, indeed, be sad if we were now to compound our initial neglect of earlier speculative indications of cell structure as a site of action of insulin by neglecting to examine the vast literature dealing with specific transport sys-

out effect. Since much of the discussion of matters such as cell membrane and transport systems is largely a matter of conjecture, there is really no experimental basis for assuming that the stimulating action of insulin is directly involved with the intrinsic properties of this particular transport system itself. Specific transport systems for many substances, as well as for sugars, are rather universal properties of cells and tissues, indeed, there may be nothing intrin-

sically unique about this particular insulin-activated system. The finding that mannose or fructose is an acceptable sugar in the insulin system of the rat, but not in the carcass of the dog, may have no relation to either insulin action or to unrecognized differences in experimental technique. Cardiac mus-

by different investigators The eviscerated carcass,[3] diaphragm,[16] gastrocnemius muscle,[16] heart,[12] aqueous chamber of the eye, and lens[11] have been used by various workers Much has been made of occasional differences in the list of sugars affected by insulin without reference to species and tissue There is general agreement in experience that

There are other considerations that caution against simple identification of insulin with the actual apparatus of cellular transport of sugars Insulin, with its own dependence on specific chemical structure for biological activity, is not the only agent that can activate the intracellular transport of a definitive group of carbohydrates The capacity of contraction of skeletal muscles to increase

been known. When in-

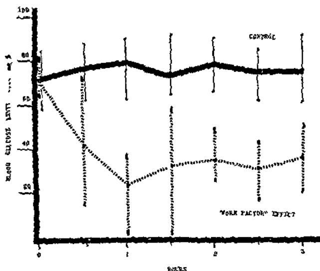
cellular entry of glucose was a limiting step in the first instance, it must also be so in the second. Since free glucose within the cell was essentially absent, greater intracellular utilization by work could not of itself lead to greater inflow of glucose. We were able to demonstrate in parallel experiments in eviscerated-nephrectomized animals that forced vigorous contraction of large masses of skeletal muscle would effect intracellular distribution of the same nonmetabolized sugars as are acted upon by insulin[21] We proposed that the glucose transport system was activated not only by insulin but by some humoral factor that was released from strenuously contracting skeletal muscles This demonstration could be achieved in

tion of severe insulin

firmed by others, but

humoral effect of working musculature[18] The action of skeletal muscle seems, indeed, to be a local effect with mild and moderate degrees of contraction With severe contraction the effect becomes generalized, manifesting itself not only in the working muscle but in the resting tissues of the animal. More strikingly, demonstration of this humoral effect of working skeletal muscle can be achieved with the use of cross-transfusion techniques Employing either an intact or an eviscerated-nephrectomized animal as the working partner, a resting, eviscerated-nephrectomized dog receives an exchange of venous blood by matched pumps in the order of 50 cc/min and is disconnected from this vascular connection at the end of a 1-hour exposure to the circulation of the working animal The resting animal, now free of any further contact with

[illegible] the amount of sugar required to maintain the normal blood level must now be doubled or even tripled Furthermore, "insulin-responsive" sugars such as D-galactose or D-xylose will exhibit an area of *distribution* far wider than that seen *in the noninsulinized resting state* *The working partner can be chronically depancreatized and* still *yield* an insulinlike *effect* Neither the *working partner nor the resting* one



[illegible]

has a source of insulin in this situation Strenuous muscle work unquestionably yields some humoral influence that not only acts on the resting tissues of

[illegible]

by insulin and by some humoral factor released from strenuously exercising skeletal muscle *itself* Although generally similar, *the precise* chemical *con-*

figuration required by the transport system can be viewed as an intrinsic property of the specific tissue of a given animal. The activation or relief of inhibition of the system would be the area of concern of such things as insulin or the humoral factor of muscular work.

Such a normally inhibited state of the transport system would be consistent with the phenomona described by Randle and Smith[22, 23] concerning the relation of adequate oxidative metabolism of the diaphragm to a diminished rate of glucose entry A variety of anoxic and chemical influences that will depress the generation of adequate high-energy-bond phosphates will lead to a freer rate of entry of glucose. This observation has been interpreted in terms of a

or teleological terms, the extremely high capacity for glucose uptake that a completely insulinized skeletal muscle and adipose cell can exhibit is meaningful only

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storage are faced with a dual functional problem, expression of rapid rates of glucose uptake in the brief postprandial period and severe inhibition of this high glucose-uptake capacity in the much longer fasting period. It would not be inconsistent with the physiological role of the sites of carbohydrate storage for inhibition of the transport system to be a major normal function of the biosynthetic activity of the cell

Dissociating insulin action in our thinking from the intimate operation of sugar transport and seeking its influence upon relieving a normal inhibition of such a built-in apparatus might lend some resolution to a variety of growing

independently of any action on glucose These are, indeed, independent data and have no direct bearing upon the data obtained with the carbohydrates These are not alternative demonstrations, and they do not reveal some indiscriminate action of insulin in allowing all manner of materials to flush into the cell Only certain amino acids share in this demonstration Their quantitative aspects, including their relations to protein and to growth, remain to be examined

in isolated

eral confirm.

action of insulin

They could again be fitted into a scheme whereby insulin and such factors

as muscle work, which parenthetically is itself a stimulus to protein synthesis, do not directly interact with transport systems themselves, but operate in some fashion to "uncover," or "reveal," or "allow" systems built into the surface of these cells to express themselves For glucose, the secondary consequences of insulin's presence or absence are extremely apparent, leading to the syndrome of diabetes. The uptake of certain amino acids could very well be involved in a similar parallel fashion, but be obscured by both the relatively slow rate of amino acid turnover as compared with carbohydrates on the one hand and the very profound effects upon protein synthesis that are attendant to the metabolic consequences of impaired uptake of carbohydrate in diabetes mellitus

The conviction that a cell surface site would explain the regulatory action of insulin on carbohydrate metabolism was derived from the behavior of a variety of sugars that served as models for glucose in response to insulin The work of many others in these last few years has served only to strengthen the feeling that there is validity in constructing a picture of regulated rates of entry of materials at the surface of the cell as the site of action of insulin There is no justification at this time, however, for any degree of rigidity in thinking about the details of such a system Just as a glucose transport system is viewed as regulating the flow of substrate to the intracellular enzymes, the activity of the transport system in turn can be regulated by insulin by affording it access to the extracellular fluid that bathes it The precise nature of the transport system itself may be a function of the particular tissue and the particular animal species, as indeed it is for analogous transport system in other tissues, where insulin is not involved In similar fashion, if insulin serves to allow a transport system to express its innate properties by "uncovering" it in some fashion, it could afford the same opportunity to adjacent transport systems for such things as certain amino acids

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REGULATION OF GLUCOSE UPTAKE IN HEART MUSCLE FROM NORMAL AND ALLOXAN-DIABETIC RATS THE EFFECTS OF INSULIN, GROWTH HORMONE, CORTISONE, AND ANOXIA*

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Introduction

The regulation of glucose uptake in muscle tissue is recognized to be of great importance for the maintenance of a normal blood glucose concentration A number of hormones, including insulin and secretions of the anterior pituitary and adrenal cortex, are known to be involved in this control In addition, some nonendocrine factors such as muscular exercise and anoxia also play a role In the present study we have attempted to make a detailed analysis of the process of glucose uptake with the object of obtaining a better understanding of how and at what point the above factors exert their effects The vari-

hypophysectomized diabetic animals

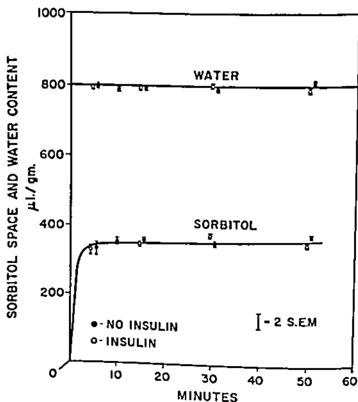
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transport of glucose through the cell wall, and third, the phosphorylation of glucose inside the cell The rate of uptake depends upon the resistance to the flow of glucose offered by each of these steps individually, but the step having the greatest resistance will be predominantly rate limiting

In order to obtain satisfactory control of the experimental conditions we used an *in vitro* muscle preparation The test object chosen was the isolated perfused rat heart A technique was developed by which a small volume of bicarbonate-buffered medium at 37° C containing glucose could be recirculated through the tissue by the normal vascular bed The muscle has no cut edges, so all substances entering the cell must pass through the membrane The viability of the preparation was excellent, as judged by its contractile activity and stable perfusion pressure Details of the procedure will be presented elsewhere[1]

bitol, which is the nonmetabolized alcohol analogue of glucose and which cannot penetrate the cell FIGURE 1 shows that sorbitol added to the medium

by insulin, alloxan diabetes, or hypophysectomy.



The rate of sorbitol distribution in this space is indicated in FIGURE 2. The curve (*open circles*) shows that sorbitol left the capillary so rapidly that about 80 per cent of the extracellular water was equilibrated

lated that the rate of transfer into the extracellular water was about 60 mg /

on glucose uptake in this muscle under ordinary conditions

Transport and Phosphorylation in Normal Heart Muscle

The control of uptake by steps 2 and 3, the transport and phosphorylation of glucose, was next considered Earlier studies, reviewed elsewhere,[2] indicated that transport and phosphorylation were distinct from each other Transport

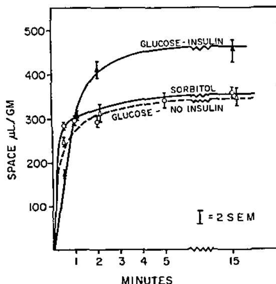


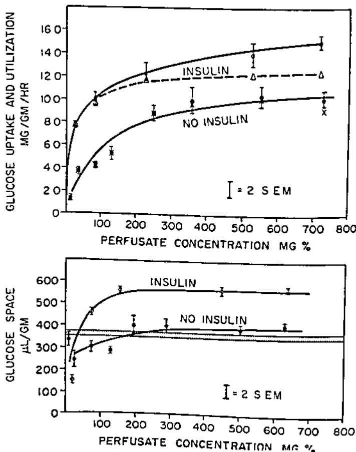
FIGURE 2 Time course of the equilibration of sorbitol and glucose between the perfusate and tissue water Hearts were perfused with 50 mg per cent sorbitol 1 C^{14} and 100 mg per cent D-glucose Insulin was added, as indicated, in a concentration of 3 μg /ml

appeared to involve a specific, reversible chemical reaction of the sugar with a membrane component This was shown by competition between glucose and a variety of sugars, including the common pentoses The product of transport across the membrane was the free sugar, which was then phosphorylated by the hexokinase system inside the cell

The rates of uptake and phosphorylation in hearts perfused at various concentrations of glucose with and without added insulin are shown in FIGURE 3.

Uptake in the Absence of Added Insulin

The "no insulin" line in the upper graph of FIGURE 3 shows the values for glucose uptake and glucose phosphorylation, which were virtually identical in the absence of added insulin. It can be seen that the curve rose with increasing concentration and showed an obvious tendency to plateau at the higher concentrations. The corresponding curve in the lower graph for the free glucose space rose slightly, but then leveled off at a value not significantly differ-



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of glucose into the cell was accelerated The transport, however, never became sufficiently rapid to exceed the capacity for intracellular phosphorylation, since no free glucose accumulated inside the cell, as shown by the fact that the glucose space did not exceed significantly the extracellular volume Thus, phosphorylation could proceed only as fast as substrate was transported, that is to say, transport was the predominantly rate-limiting step over the entire range of glucose concentration

Uptake in the Presence of Insulin

As shown by the uppermost lines of the upper graph, glucose uptake (*solid line*) and phosphorylation (*dashed line*) rose more rapidly with insulin, and phosphorylation reached a plateau at about 12 mg /gm /hr Corresponding to this plateau, free glucose accumulated in large amounts inside the cell, as shown in the lower graph by the rise in glucose space far above the extracellular volume The interpretation was as follows

The uptake of glucose and consequently the transport of sugar into the cell were accelerated by insulin The acceleration of transport was so great that, above 50 mg per cent, the capacity for phosphorylation was exceeded, and free sugar accumulated inside the cell The curves show that the insulin effect on uptake consisted of 2 components, both of which were secondary to transport acceleration In the physiological range of glucose concentration the acceleration of transport led to a substantial increase in phosphorylation At high glucose concentrations however, phosphorylation was already near capacity in

[illegible]

stimulation It is also clear that, once the cell was saturated with free sugar, the rate of uptake was limited by the phosphorylation step

Kinetics of Transport

Since uptake in the absence of insulin was limited by transport, the curve of uptake provided a picture of the kinetics of inward transport as a function of

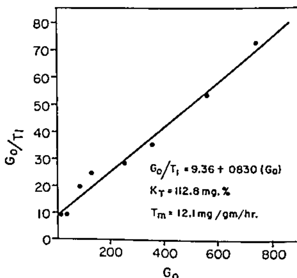
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nated K_t on the graph, was about 110 mg per cent glucose, that is 6×10^{-3} M, and the transport maximum was 12 mg /gm /hr.

Effect of Anoxia in Normal Hearts

Glucose uptake Randle and Smith[5, 6] have shown recently that glucose uptake in muscle is accelerated by anoxia This important observation has been studied further by Morgan *et al.*[7] Some representative data obtained with the perfused rat heart are shown in FIGURE 5

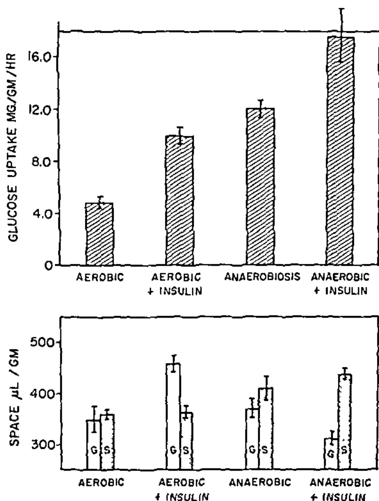
stimulated glucose uptake in the absence of insulin. Since transport was rate-limiting for uptake, it may be concluded that anoxia stimulated the transport



cose suggested an increase in phosphorylation capacity induced by the anoxic state. This increase could be shown in the presence of insulin. Despite very high rates of uptake (hence rapid transport), no free sugar accumulated in the cell, and the limit of phosphorylation capacity was not reached Under corresponding aerobic conditions, phosphorylation became limiting at a much lower level of uptake

L-Arabinose transport The effect of anoxia on transport was also studied, using the glucose analogue L-arabinose This pentose crosses the cell membrane by the same mechanism as glucose, as shown by competitive inhibition ... but is not detectably phosphorylated.[8] It ...

measure transport by determining the efflux of L-arabinose from the intracellular fluid of hearts that had accumulated a large amount of sugar during a preliminary perfusion As shown in FIGURE 6, the rate of outward transport was a linear function of concentration under all conditions tested In comparison to the control, anoxia caused approximately a twofold acceleration of trans-



port. In the heart already treated with insulin, in which transport was accelerated about fourfold, anoxia had no additional effect. These data show that the transport effect of anoxia was much smaller than that of insulin The

insulin on transport under anaerobic condition.

Effect of Alloxan Diabetes on Glucose Uptake

Reduced rate of transport The next series of experiments was concerned with the effect of alloxan diabetes on glucose uptake. The upper panel of FIGURE 7

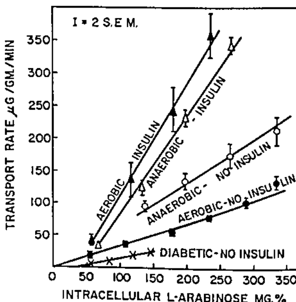
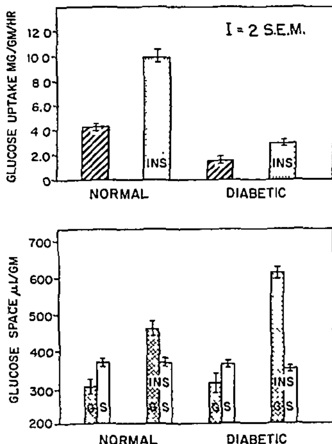


FIGURE 6 The effects of insulin, anoxia, and alloxan diabetes on the transport of arabinose out of the isolated perfused heart (see text for description)

shows that alloxan diabetes greatly reduced glucose uptake Uptake was on slightly accelerated with the addition of insulin in the short time of these e periments. The glucose spaces are shown in the lower panel of FIGURE 7. Tl glucose space was less than extracellular in the diabetic, indicating that no fr sugar was present inside the cell It could be concluded, therefore, that tran port was rate limiting for uptake in the diabetic and was much reduced. Th confirmed our earlier observations in the whole animal[8, 9] and could also l demonstrated in experiments employing the nonmetabolized glucose analog L-arabinose, the transport rate of which was found to be very low (see FIGU 6).

Depression of phosphorylation. The addition of insulin to the diabetic hea stimulated transport into the cell, as shown by the accumulation of intracell lar free glucose. Despite the abundance of sugar, however, the uptake

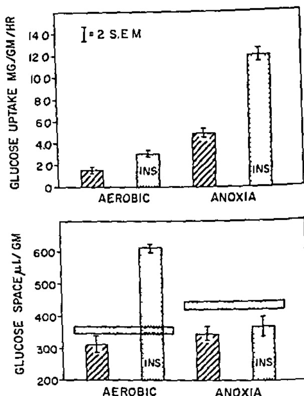
glucose rose very little Since phosphorylation was clearly rate limiting under these conditions, it could be concluded that phosphorylation in the diabetic muscle was also *impaired.*



It was also clear that insulin had no immediate major effect on the phosphorylation rate in diabetic muscle, in line with the result obtained with normal tissue

Effect of anoxia The effect of anaerobiosis on glucose uptake by diabetic hearts is shown in FIGURE 8 Glucose uptake was accelerated in *the absence*

of insulin. Since transport was rate limiting, as shown by the absence of in-
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tion very greatly,
free sugar in the cell. This increase was so great in fact that transport remained the rate-limiting step

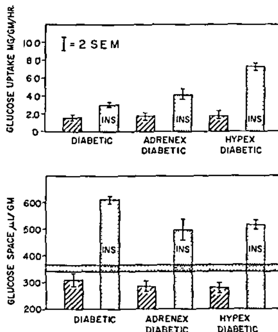


In other experiments it was found that a return to aerobic conditions after a period of anoxia resulted in the prompt reappearance of a depressed phosphorylation rate.

Effect of the Pituitary and Adrenal Secretion on Uptake by the Diabetic Muscle

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it appeared that pituitary secretions did not have any striking effect on the transport rate. When insulin was added, the glucose uptake rose appreciably above that in the simple diabetic Since phosphorylation was the limiting step, it could be concluded that removal of the pituitary resulted in an increased phosphorylation capacity In other words, the pituitary secretion was in part responsible for the depressed phosphorylation of the diabetic muscle



[illegible]
450 mg per cent

The effect of hypophysectomy on the glucose uptake by the heart of normal rats was also investigated in some detail, and the results will be published shortly. It may be noted here that glucose uptake was appreciably lower than normal, and that this depression was due to a diminished rate of transport Phosphorylation capacity in the presence of insulin was about the same as in the normal tissue

The effect of adrenalectomy on the glucose uptake by the diabetic heart was not striking (see FIGURE 9) There was no rise in uptake in the absence of insulin and only a small rise in its presence These data suggested that the

adrenal secretions did not have any effect on transport, and that their inhibitory effect on phosphorylation was smaller under these conditions.

Growth Hormone and Cortisone

Since removal of the pituitary or adrenal appeared to improve the phosphorylation capacity in the diabetic muscle, the effect of growth hormone and/or cortisone readministration was studied, using hypophysectomized diabetic rats These animals were treated either with growth hormone alone

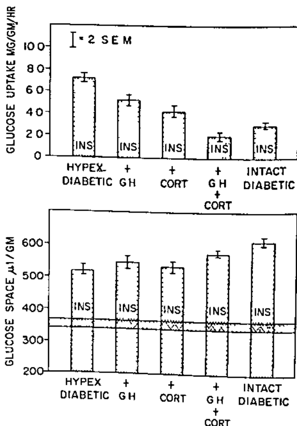


FIGURE 10 The effect of growth hormone and/or hydrocortisone on the glucose uptake

700, growth hormone and hydrocortisone, 670 mg per cent

cortisone alone, or with both hormones together twenty-four hours before sacrifice In all cases the hearts were perfused in the presence of insulin in order to establish a sufficiently rapid transport rate to accumulate intracellular free sugar and to make phosphorylation rate limiting for uptake

The results are shown in FIGURE 10 Treatment with growth hormone alone led to a small depression of phosphorylation, as seen by the reduced uptake in the presence of abundant intracellular free glucose Similarly, cortisone alone had a distinct inhibitory action Both agents together were much more effective than either alone, and they depressed phosphorylation to the level of the simple diabetic

Discussion

It was concluded from the present analysis of glucose uptake that the extracellular transfer of glucose from within the capillary to the cell was so rapid that the process did not have an important controlling influence on the overall rate of uptake Some caution is necessary, however, in extending this conclusion, obtained with cardiac tissue, to other muscles, particularly resting skeletal muscle, where the circulatory system is largely shut down and is intrinsically sparser than in the heart In fact, it would appear likely, as suggested earlier,[3] that accelerated transfer due to opening of the capillary bed may contribute to the increased glucose uptake by skeletal muscle with muscular exercise. It is also not clear at present whether or not extracellular transfer is affected by hormonal factors Any such effects, if present, would be particularly important physiologically at low blood sugar concentrations, where the possibility is greatest that extracellular diffusion could become a rate-limiting step

The studies with the heart preparation presumably reflect qualitatively the basic acceleratory action of insulin on the sugar transport process in muscle tissue in general, as first described by Levine and her associates[10, 11] Our present work also shows the lack of any large, direct effect of insulin on phosphorylation From a quantitative point of view, however, the insulin effect may be somewhat different in other muscles, particularly resting skeletal muscle For example, it has been difficult to show accumulation of free sugar in the resting gastrocnemius with insulin[3, 13, 14] under conditions in which it accumulates readily in the heart and diaphragm This fact presumably reflects a different balance in the rates of extracellular transfer, membrane transport, and phosphorylation such that the transport rate, although accelerated by insulin, does not readily exceed the phosphorylation capacity

The observation that glucose transport as a function of concentration follows Michaelis-Menten kinetics fits well with the concept that transport involves a chemical reaction between glucose and a membrane constituent, as reviewed elsewhere[2] It is of interest that the K_m for transport in muscle is of similar magnitude to that found in the erythrocyte by LeFevre[15] The K_m value is such that uptake is particularly sensitive to changes in blood glucose concentrations in the physiological range

With regard to anoxia, the question of whether transport acceleration is due

to a nonspecific loss of membrane integrity has been examined The following four points, presented elsewhere in detail,[5,7] suggest that this is not the case. first, it has been shown that competition between sugars is preserved under anaerobic conditions, second, phloridzin strongly depresses transport and is as effective anaerobically as aerobically, third, anoxia does not accelerate transport in the insulinized muscle, as shown in FIGURE 6 (an effect would be expected if pores were created for diffusion); and fourth, the anoxia effect on transport and phosphorylation is promptly reversed with a return to aerobic conditions In this connection we also found that the increase in sorbitol space with anoxia, seen in FIGURE 5, was returned promptly to normal by restoration of an oxygen atmosphere. This result indicated that the increase in sorbitol space was due to expansion of the extracellular volume and not to a leaky membrane

The effects of anoxia are important in at least three respects: first, the effects [illegible] requirement of the diabetic individual [illegible] exercise. Second, the anoxia effect contributes to our understanding of the Pasteur effect, as pointed out by Randle and Smith.[5, 6] The acceleration of transport is particularly important in the case of muscle, since transport is the rate-limiting step for uptake in this tissue, as contrasted with most other tis[illegible] This observation suggests the pos[illegible] of course, that insulin itself may act primarily within the cell, and that more rapid transport is secondary to intracellular metabolic changes, but whether or not this is the case has not been established. While insulin and anoxia both accelerate transport, it is clear that they differ in their actions in two important regards The insulin effect on transport is much greater than that of anoxia (see FIGURE 6) and, conversely, anoxia strongly stimulates glucose phosphorylation, whereas insulin has little or no such immediate action.

The analysis of glucose uptake in the diabetic muscle shows that the first physiological defect is a reduced rate of transport, this result is in agreement with earlier studies in the whole animal[8] The second defect, a depressed phosphorylation, was not appreciated in our earlier work, but has been suggested by the studies of Kipnis *et al*[14] and is apparent in the present experiments The relative importance of the transport and phosphorylation im[illegible] essed transport is most important, since transport [illegible] sence of insulin, however, when transport is accelerated the phosphorylation defect is most important, since phosphorylation becomes rate limiting under these conditions The fact that insulin administration causes no acceleration of phosphorylation, at least in the short

period of the present experiments, may account for some of the insensitivity to insulin observed frequently in severe diabetes with the initiation of treatment with the hormone Presumably, the phosphorylation defect disappears with prolonged insulin treatment, but this has not been tested experimentally as yet in muscle

The marked accelerating effect of anoxia, particularly in the presence of insulin, further emphasizes the importance of the depressed phosphorylation in diabetic muscle. In view of the high rates obtained with anoxia, it is clear that the phosphorylation impairment is not due to a deficiency of enzyme This is in contrast to studies in diabetic liver, where marked alterations in enzyme levels have been found.

The contribution of the pituitary and adrenal glands to the metabolic defect [illegible] factors affect the phosphorylation step, and insulin effects are confined to transport In agreement with this view, hypophysectomy or adrenalectomy does not improve the depressed transport in the diabetic, suggesting that the block at this point is due simply to the absence of insulin This block remains rate limiting for uptake until insulin is given Transport acceleration then shifts the rate-limiting step to the phosphorylation level Adrenalectomy of the diabetic raises phosphorylation capacity slightly, and hypophysectomy has a marked effect on it This increase in capacity makes room for a much larger effect of insulin on the glucose uptake Injection of growth hormone and/or cortisone in the hypophysectomized diabetic depresses phosphorylation. Either hormone alone has some effect, but the largest response is obtained with both together This observation is in accord with many studies showing a synergism of these hormones in inducing a diabetic state

Summary

The process of glucose uptake has been analyzed in the perfused isolated rat heart The extracellular transfer of glucose is very rapid and does not limit glucose uptake in the normal or diabetic tissue The transport of sugar through the membrane is the rate-limiting step in normal and diabetic hearts The rate is reduced in the diabetic and is not accelerated by hypophysectomy or adrenalectomy Transport is stimulated, on the other hand, by anoxia in both the normal and diabetic muscle. At the same time anoxia markedly increases the phosphorylation capacity Insulin stimulates transport to a much greater extent than anoxia, but does not immediately increase phosphorylation capacity

port appear to be insulin and the level of aerobic metabolism. Factors regulating phosphorylation are pituitary (growth hormone) and adrenal (hydrocortisone) secretions and the presence or absence of aerobic metabolism.

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EFFECTS OF INSULIN ON ADIPOSE TISSUE*

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Albert E Renold

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The purpose of this report is to summarize observations made in the Baker Clinic Research Laboratory on the action of insulin on adipose tissue We have become impressed by the sensitivity of this tissue to insulin, as well as by the magnitude of the response obtained Indeed, it appears to us that adipose tissue may well represent the major site of insulin action in the intact organism This reappraisal of the metabolic importance of adipose tissue is also in keeping with the extensive studies carried out in other laboratories, notably those of Wertheimer and Shapiro,[1] Hausberger,[2] Favarger,[3] and Stetten [4] In general, it may be stated that the role assigned to this tissue is rapidly changing from that of an inert storage site to that of an extremely active and labile system intimately concerned with the synthesis, storage, oxidation, and release of body fats.

In the early studies, adipose tissue was shown to respond to insulin by an accelerated glucose uptake,[5] by increased glycogen synthesis both *in vivo*[6,7] and *in vitro*,[8] by a marked elevation of the respiratory quotient to values greater than unity[9] and, in the hands of some investigators, by an increased oxygen consumption.[10] More recently the use of carbon-14 and deuterium-labeled compounds has provided a more quantitative appreciation of the insulin effect FIGURE 1 summarizes the average of several experiments with glucose randomly labeled with carbon-14 The marked insulin effect is easily seen, namely, a sixfold increase in the oxidation of glucose to CO_2 and an approximately tenfold increase in fatty acid synthesis Glycerol synthesis is doubled and glycogen synthesis is increased almost twentyfold, although these metabolic fates are quantitatively less important in the total disposition of glucose carbon

Since added insulin produces such a marked stimulatory effect, adipose tissue from animals that are presumably insulin deficient should exhibit a decreased metabolism of glucose. FIGURE 2 compares the disposition of labeled glucose in normal and alloxan-diabetic rats, with and without insulin added to the incubation flask The failure of insulin *in vitro* to elevate the decreased metabolism of adipose tissue from the diabetic animal to that of the normal one treated with insulin may be due to other secondary or adaptive phenomena, however, the markedly decreased basal level of glucose metabolism in adipose tissue from the diabetic organism is evident

Milstein[11] first reported a much greater increase in the oxidation of the first

carbon (carbon-1) of glucose when compared to the last carbon (carbon-6) after insulin stimulation It was therefore postulated that insulin selectively stimulated the pathway responsible for the preferential oxidation of glucose carbon-1, namely, the phosphogluconate-oxidative pathway. However, sub-

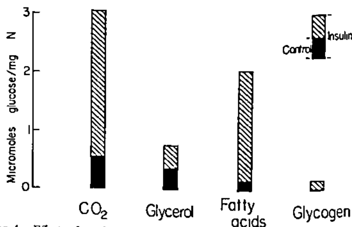


FIGURE 1 Effects of insulin on the metabolism of glucose-U-C[14] by rat adipose tissue The insulin concentration is 0 1 U /ml

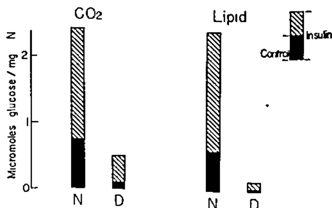


FIGURE 2 Effects of insulin on the metabolism of glucose-U-C[14] by adipose tissue from normal (N) and from alloxan-diabetic (D) rats The insulin concentration is 0 1 U /ml.

sequent experiments by Winegrad[12] in our laboratory including recovery of
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Again, this was true either with or without insulin stimulation. From the

data it would appear that glucose is metabolized equally by two pathways, and that insulin stimulates both pathways to the same extent or, more probably, that insulin stimulates the conversion of medium glucose to intracellular glucose-6-phosphate, the metabolic precursor from which both the glycolytic (or Emden-Meyerhof) and the phosphogluconate-oxidative pathways diverge

Acetate is also utilized by adipose tissue for fatty acid synthesis, as are many other substrates In FIGURE 4 further evidence is presented to indicate that the insulin effect appears to be mediated primarily by increased availability of glucose to intracellular metabolism [12] Adipose tissue was incubated with acetate-1-C^{14}, and the incorporation of label into lipid and CO_2 was measured

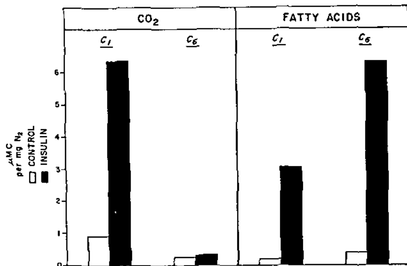


FIGURE 3 Effects of insulin on the metabolism of glucose-1-C^{14} and glucose-6-C^{14} by rat adipose tissue *in vitro* Bicarbonate buffer, 3 hour incubation, 20 mM/l glucose, 0 1 U insulin Reproduced by permission from *The Journal of Biological Chemistry* [13]

with and without added insulin and with and without added glucose No effect is observed with insulin alone Glucose alone doubles acetate incorpora-

an increased conversion of medium or circulating glucose into tissue glucose-6 phosphate (note that our system does not differentiate between a process involving increased permeability or translocation and one involving phosphorylation), a similar pattern of glucose metabolism should be obtained whether the increase is secondary to insulin or secondary to increased glucose concentration in the medium Recent experiments were performed whereby the concentration of glucose and the concentration of insulin were varied independently.[13] FIGURE 5 demonstrates the relation of glucose-1-C^{14} and glu-

carbon (carbon-1) of glucose when compared to the last carbon (carbon-6) after insulin stimulation. It was therefore postulated that insulin selectively stimulated the pathway responsible for the preferential oxidation of glucose carbon-1, namely, the phosphogluconate-oxidative pathway. However, sub-

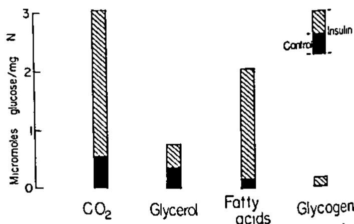


FIGURE 1. Effects of insulin on the metabolism of glucose-U-C^{14} by rat adipose tissue. The insulin concentration is 0.1 U./ml.

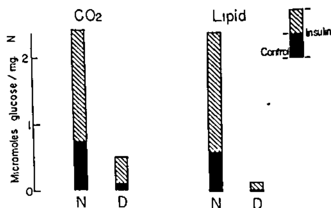


FIGURE 2. Effects of insulin on the metabolism of glucose-U-C^{14} by adipose tissue from normal (N) and from alloxan-diabetic (D) rats. The insulin concentration is 0.1 U./ml.

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Again, this was true either with or without insulin stimulation. From the

cose-6-C^{14} metabolism to varying concentrations of glucose in the medium Again, the C-1/C-6 ratio in fatty acids remained constant at about 0 5. Com-

tained constant at 20 mM/l (360 mg per cent), whereas insulin concentration was varied from 0 to 100,000 μU/ml. The similarity of the two patterns shown in FIGURES 5 and 6 clearly suggests that the increased metabolism induced by increasing concentrations of glucose is identical to that induced by insulin

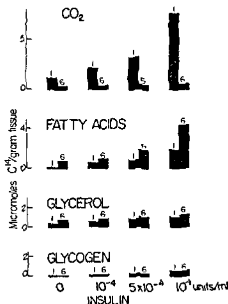
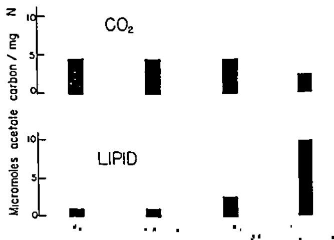


FIGURE 6 Effect of varying concentrations of insulin on the metabolism of glucose-1 C and glucose 6-C^{14} by adipose tissue The concentration of glucose is 20 mM/l throughout

action. This is further support for a unifocal hypothesis of insulin action resulting primarily in facilitation of the conversion of extracellular glucose to intracellular glucose-6-phosphate

respectively).



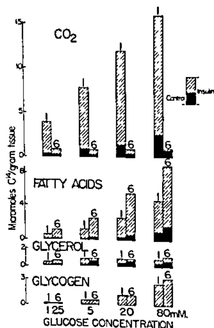


FIGURE 5 Effects of varying concentrations of glucose on the metabolism of glucose-1-C14 and glucose-6-C14 by adipose tissue with and without added insulin (0.1 U./ml.) The numerals 1 and 6 at the top of each column refer to the specifically labeled glucose used

cose-6-C^{14} metabolism to varying concentrations of glucose in the medium Again, the C-1/C-6 ratio in fatty acids remained constant at about 0 5 Comparison of the oxidation of glucose-carbon-1 with that of carbon-6 reveals that the increment is mainly from carbon-1. FIGURE 6 reveals an entirely similar pattern, although in these experiments the concentration of glucose was maintained constant at 20 mM/l (360 mg per cent), whereas insulin concentration was varied from 0 to 100,000 μU/ml The similarity of the two patterns shown in FIGURES 5 and 6 clearly suggests that the increased metabolism induced by increasing concentrations of glucose is identical to that induced by insulin

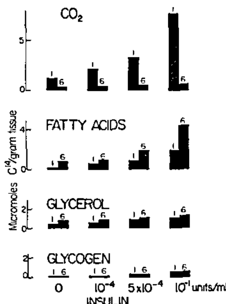


FIGURE 6 Effect of varying concentrations of insulin on the metabolism of glucose 1 C and glucose-6-C^{14} by adipose tissue The concentration of glucose is 20 mM/l throughout

sensitive Mannose was metabolized as actively as glucose, fructose and galactose less so (approximately one fifth and one twentieth the rate of glucose, respectively).

Recent and preliminary experiments by Y. Dagenais and G Zahnd in our laboratory suggest an interesting effect of potassium on glucose metabolism by adipose tissue In the total absence of this cation (FIGURE 7) glucose oxidation,
presence
effect
cance of these findings

The synthesized fatty acids are deposited in the tissue; only a trace is released into the medium even in the presence of albumin as an acceptor Partition of the lipids in the tissue itself shows that 98 per cent or more of the labeled fatty acids are present as esters However, incubation with labeled fatty acids such as palmitate reveals the process of rapid exchange of tissue fatty acids

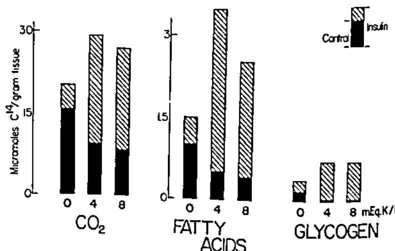


FIGURE 7 Effects of varying concentrations of potassium on the metabolism of glucose-1-C^{14} by adipose tissue with and without added insulin (0 1 U /ml)

with those in the medium[14] In addition, if the medium fatty acid concentration is raised above 0 7 mEq /l, the tissue shows a net fatty acid uptake in addition to still greater exchange of tissue fatty acids with those in the medium

The glycerol used to esterify the fatty acids is synthesized *de novo* from glucose Other experiments have shown, in confirmation of those of Shapiro *et al.*,[15] that added labeled glycerol is utilized to a very limited degree In the control tissue, the synthesis of glycerol is 17 times greater, mole for mole, than is the rate of synthesis of labeled fatty acids. In the presence of insulin, the relative turnover of glycerol is less, but still 3 times that of the fatty acids. This turnover is more evidence for the great metabolic activity of what has long been considered an inert tissue.

Recently, growth hormone[16, 17] and epinephrine[18] have also been shown to increase glucose uptake by normal adipose tissue *in vitro*. The patterns of

metabolism resulting from the presence of these two hormones and also that resulting from the addition of ACTH[19] differ markedly from that due to in- [illegible] epinephrine, as in the case of insulin, studies with glucose labeled in either the 1, 2, or 6 positions have permitted an evaluation of the relative activities of several metabolic pathways. Schematically (FIGURE 8), when one compares the metabolism of insulin-stimulated tissues to that of control tissues, there is a simultaneous increase in both the glycolytic and the phosphogluconate-oxidative pathways, as stated previously, with predominant metabolism of glucose carbon to fatty acids and to CO_2, the latter being derived from the phosphogluconate-oxidative pathway, as well as from the decarboxylation of

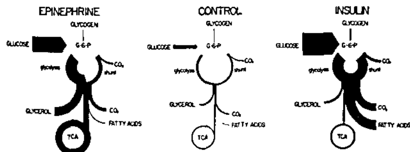


FIGURE 8 Diagrammatic representation of glucose metabolism by rat adipose tissue incubated without the addition of hormones and in the presence of either insulin (0 1 U /ml) or epinephrine (0 1 mM/l) The thickness of the bars indicates the relative rates of metabolism The data upon which this diagram is based were derived from several studies

pyruvate to acetate The increased uptake after epinephrine, on the other hand, is associated primarily with increased glycolytic activity and glyceride-glycerol synthesis Fatty acid synthesis is much less and, of the small quantity synthesized, much is released into the medium along with active release of unlabeled unesterified fatty acids Recent experiments have also shown a simultaneous outpouring of formaldehydogenic substance (presumably glycerol) from the tissue Insulin and epinephrine added together increase the glucose uptake to an extent greater than that resulting from either alone, but the resulting metabolic pattern is identical to that produced by epinephrine alone. That epinephrine leads to marked glycogenolysis in adipose tissue obtained from fasted-refed animals has recently been established both *in vivo* and *in vitro* and by chemical as well as by histochemical techniques[20] In the case of growth hormone, a specific stimulation of the uronic acid pathway has been suggested as the best explanation for the specific increase in the oxidation of carbon-6 by adipose tissue[17] On the other hand, the *in vitro* addition of hydrocortisone (25 μg /ml) to adipose tissue[21] does not alter either its baseline

metabolism or the effect of insulin on lipogenesis from glucose labeled in carbon-6, as shown in FIGURE 9

Finally, it should be stressed that the effect of insulin on adipose tissue is obtained with concentrations well within the physiological range. In fact, if one accepts present estimates of the concentration of insulin in normal human serum as approximately 100 μU./ml., it is noteworthy that alterations in the metabolism of glucose by adipose tissue *in vitro* are obtained with one tenth of this physiological concentration of the hormone. This sensitivity has led us to employ adipose tissue incubated in the presence of glucose-1-C^{14} for the purpose of measuring insulinlike activity in serum.[22]

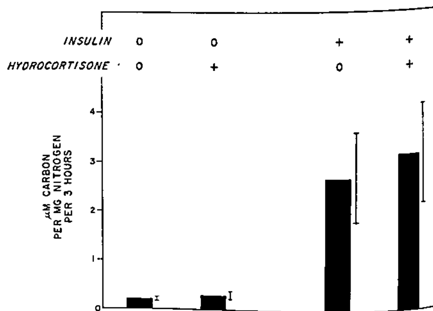


FIGURE 9 The effect of hydrocortisone added *in vitro* (25 μg/ml) on lipogenesis from glucose 6-C^{14} by rat adipose tissue

organism.

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EFFECTS OF INSULIN AND TOLBUTAMIDE ON BLOOD GLUCOSE ENTRY AND REMOVAL RATES*

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The Institute for Cancer Research, The Albert Einstein Medical Center and The Home for the Jewish Aged, Philadelphia, Pa.

During the past several years we have been conducting experiments, using C^{14}-labeled glucose, with the object of obtaining information concerning the factors involved in the regulation of entry and removal of the blood glucose. As a result of these studies[1,2] we were led to the conclusion that insulin exerts its hypoglycemic action not only by accelerating the removal of glucose from the blood, but also by suppressing hepatic glucose output. These studies left in doubt the quantitative importance of this hepatic action of insulin, but indicated that under certain circumstances it might be of major significance in blood sugar regulation We shall describe briefly the principles of our procedures and present certain experiments that lead us to believe that insulin suppresses the hepatic output of glucose.

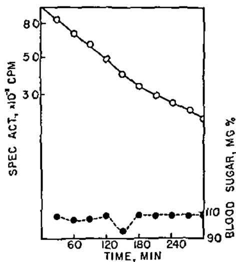
The procedure used represents an extension of a familiar isotope-tracer technique employed previously for a study of glucose turnover in rats, dogs, and man[1,2] A trace dose of glucose uniformly labeled with C^{14} is injected, and blood samples are removed at frequent intervals for determination of blood sugar content and specific activity.

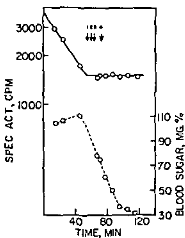
No attempt is made in this brief report to describe the details of our methods of determination of specific activities and of calculations; all of these have been published An experiment illustrative of many thus far conducted in normal postabsorptive human subjects is shown in FIGURE 1, which illustrates the typical behavior of the blood glucose level and specific activity in a normal 16-hour-fasted human

There is thus a rather precise balance between entry and removal of the blood glucose, the rates of which can easily be estimated from the first-order reaction-rate expression

When insulin is injected, the blood sugar falls, then returns to normal This hypoglycemic effect must be due either to a suppression of entry or to an enhancement in removal, or to both If the hypoglycemia is due to an acceleration of removal, the specific activity will continue to fall at least as rapidly as before, since the incoming glucose molecules are diluting a pool that continuously becomes smaller, however, if the drop is due in some part to an inhibition of entry, the specific activity will fall less rapidly, since the blood glucose will be more slowly diluted by new, unlabeled molecules. The behavior of the specific activity curve when hepatic glucose output ceases may be seen in FIGURE 2 In this experiment a normal dog was submitted to hepatectomy after a portal caval anastomosis. During the period of the operation the specific activity of the blood glucose dropped exponentially but "plateaued"

* The work reported in this paper was supported in part by grants from the Public Health Service, Bethesda, Md , The American Cancer Society, Inc , New York, N Y , and the United States Atomic Energy Commission, Washington, D C


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BLOOD SUGAR, MG %
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when the hepatic circulation was occluded and remained constant until the dog died with a blood sugar level of 30 mg /100 ml.

The type of evidence that points to an hepatic action of insulin is shown in FIGURE 3 This experiment was conducted with a fasted, normal human, a resident of a local home for the aged Exactly similar results were obtained

at the time indicated by the arrow, insulin was injected intravenously in dosage of 1 u /kg At short intervals thereafter, samples of blood were again

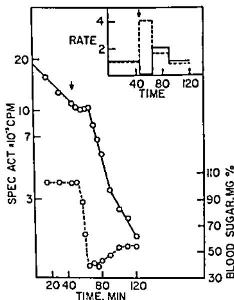


FIGURE 3 Hepatic action of insulin in a fasted normal human See text

removed for determination of the level and specific radioactivity In this experiment, which is illustrative of many thus far carried out, there is exponential drop in the blood sugar specific activity preceding the insulin

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the specific activity again resumes its downward trend We interpret the findings as indicating that, during this immediate postinsulin period, labeled glucose molecules cease to enter the blood stream. Since the liver generally recognized to be the only organ contributing glucose to the blood,

assume that this effect is brought about by a suppression of hepatic glucose output. However, the method is not designed to locate the source of the inflowing glucose, but only its occurrence.

From the data thus obtained we can estimate rough approximations of inflow and outflow rates during these various intervals preceding and following insulin administration,[1]
FIGURE 3, where the so
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estimations. In view of the large number of assumptions required in such

ml/min., second, immediately after insulin injection the rate of entry drops essentially to zero, while the removal rate increases about threefold, and, in the *third phase, entry recommences and the removal rate drops, each to approach* the preinsulin values

These results, which point to an immediate action of insulin in suppressing

groups did not regard this effect as important in its hypoglycemic action On the other hand, Ashmore *et al*[9] failed to observe plateaus in similar experiments *in rats*, and though *they did observe such plateaus in experiments with dogs*, they were unable to confirm a suppression of hepatic glucose output by portal-hepatic sampling techniques (personal communication from J Ashmore). Tarding and Schambye,[10] using both tracer methods and portal-hepatic sampling, found a suppressive action of tolbutamide, but not of insulin, on hepatic glucose output

It is now appropriate to discuss our studies with the oral hypoglycemic agent, tolbutamide There is already much evidence to show that this, as

insulin, there was a plateauing in specific activity following its intravenous injection, which coincided with the induction of hypoglycemia and terminated *at the low point in blood glucose level This finding is readily explainable in* terms of an insulinogenic action of tolbutamide, with a consequent suppression of hepatic glucose output However, when estimations are made of glucose entry and removal rates from these data we find, as shown in the insert, that there is suppression of entry, but essentially no increased removal

These findings, which
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otherwise, the experiments were conducted exactly as were the previous ones. Under these conditions two features are noteworthy, as shown in FIGURE 5. First, we observed an extremely long plateau in specific activity, lasting as

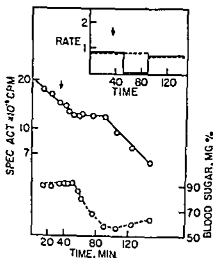


FIGURE 4. Effect of tolbutamide in the normal human. See text.

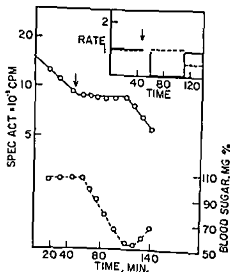


FIGURE 5. Action of subcutaneous insulin on the release of hepatic glucose. See text.

long as 1 hour, in contrast to 10 to 20 min when injected intravenously in a single dose Second, as shown in the inset, the suppression of hepatic glucose output occurs apparently without an increased peripheral utilization Other investigators also have observed recently that the slow introduction of insulin may result in hepatic effects without peripheral effects, these investigators include, notably, Craig *et al*,[16,17] Dulin and Johnston,[18] and Madison and Unger[19]

In a very recent paper Berson *et al*[20] pointed out that our values for initial glucose turnover may be too high, owing to incomplete mixing of the labeled glucose with the body pool during the preinsulin equilibration period For this reason the lack of a peripheral effect may be fortuitous We ourselves have to recognize the justice of this criticism In long-term turnover experiments we avoided the uncertainty of incomplete mixing and have indeed obtained somewhat lower initial fasting turnover rates in humans The results of 7 such recent experiments are shown in TABLE 1 Preinsulin turnover rates

TABLE 1
EFFECT OF SUBCUTANEOUS INSULIN INJECTION ON BLOOD GLUCOSE ENTRY AND REMOVAL IN MAN
(Values are in mg /100 ml blood/min)

Condition	Insulin dose μ/kg	Preinsulin turnover	Insulin, entry	1 hour removal
Nondiabetic	0 18	0 87	0	0 9
	0 14	1 0	0	1 0
	0 15	0 90	0 3	0 9
	0 10	0 95	0 7	1 2
	0 11	0 5	0 2	0 9
Diabetic	0 13	1 4	0	0 9
	0 10	0 7	0	0 6

are shown in column 2, and in columns 3 and 4 are given the rates of inflow and outflow calculated over the period, usually about 1 hour, during which the blood sugar fell to its lowest point The values for turnover ranged from 0 5 to 1 4 mg /100 ml /min in the series of experiments carried out in 5 normal and in 2 mildly diabetic humans in which at least 80 min were allowed for equilibration before insulin injection In the first 3 experiments there was no appreciable change in glucose removal rate, while entry rate during the period of insulin injection dropped to zero in 2, and to 0 3 in the third In 2 experiments the removal rate increased somewhat, and in one of these the entry dropped, but did not stop entirely In the 2 diabetics, who were very mild cases, the blood glucose removal rate did not increase, and the the entry dropped to zero

We wish to emphasize something that we have repeatedly stressed in the past We do not regard these replacement rates as anything but rough approximations What we regard as of significance is the plateauing of the specific activity curves, which tell us that glucose is not entering the blood and, in turn, allow us to estimate that the blood sugar drop is not due to a markedly increased removal rate

Without denying an important role to insulin in promoting the utilization of ... peripheral tissues, we feel that the data here presented, together ...

some hepatic action of insulin. It happens that our experiments ... that the hepatic effect is consistent and that, under certain circumstances, it may have been dominant in the resulting hypoglycemia. The same situation ... been using portal and ...

of the others. Under the circumstances it is probably overoptimistic ... better agreement in dealing with the action of a substance such as insulin whose action on the liver has been controversial ever since its discovery.

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INFLUENCES OF GLUCOSE LOADING AND OF INJECTED INSULIN ON HEPATIC GLUCOSE OUTPUT*

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Whether insulin acts immediately on the liver to reduce its rate of glucose output is a matter of great current interest. From the theoretical aspect, the answer given to this question has serious implications with respect to hypotheses concerning the mechanism of insulin action. In particular, an increase in permeability to glucose of the hepatic cell membrane should increase rather

flow and not to decrease it

other hand, to differences in interpretation and calculation.

The Body Glucose Pool

If the glucose pool were as we should like to define it rather than as it actually is, it would consist of glucose in aqueous solution in a well-stirred space

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who have studied the influence of insulin on hepatic glucose output

In FIGURE 1 the letter V represents the unchanging volume of plasma and

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* The work reported in this paper was carried out at the Brookhaven National Laboratory under the auspices of the United States Atomic Energy Commission, Washington, D. C.

cose, have concluded that the value of V does not change to an important extent during insulin action. Of the other letters appearing in FIGURE 1, A_t represents the total number of microcuries of C^{14} glucose in the pool at time t, R_1, and R_2 the amount (grams) of glucose carbon per minute entering and leaving the pool at time t, and F the amount of C^{14} glucose ("weightless" amount) in microcuries per minute being given by constant infusion (F is zero in those experiments called "single injection" experiments). The specific activity of the glucose in the compartment may be designated i_t and is equal to the ratio

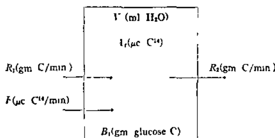


FIGURE 1. A diagram representing the conventional model for the dog body glucose pool. For explanation of the symbols see text.

A_t/B_t when the specific activity is expressed as μc C^{14} per gram of glucose carbon.

$$\text{Since } A_t = B_t i_t$$

$$\frac{dA_t}{dt} = B_t \frac{di_t}{dt} + i_t \frac{dB_t}{dt} \tag{1}$$

Also, it can be seen by inspection that the rate of change of the total glucose present in the compartment depends on the two flow rates R_1 and R_2

$$\frac{dB_t}{dt} = R_1 - R_2 \tag{2}$$

Similarly, it can be seen by inspection that the rate of change of the total C^{14} present in the pool depends on F (the rate of C^{14} infusion) and on the product of R_2 (outflow rate) and $i_{(t)}$ (the specific activity of the glucose in the compartment)

$$\frac{dA_t}{dt} = F - R_2 i_t \tag{3}$$

Combining EQUATIONS 1, 2, and 3, there are obtained equations for R_1 and R_2:

$$R_1 = \frac{F - B_t \frac{di_t}{dt}}{i_t} \tag{4}$$

$$R_2 = R_1 - \frac{dB_t}{dt} \tag{5}$$

For numerical approximations these equations can be used in the forms.[9]

$$R_1 = \frac{F - \left(\frac{B_{(t)} + B_{(t+\Delta t)}}{2}\right)\left(\frac{i_{(t+\Delta t)} - i_{(t)}}{\Delta t}\right)}{\frac{i_{(t)} + i_{(t+\Delta t)}}{2}} \tag{4a}$$

$$R_2 = R_1 - \frac{B_{(t+\Delta t)} - B_{(t)}}{\Delta t} \tag{5a}$$

When F is zero as in the "single-injection" procedure, integrating EQUATION 5 then combining it with EQUATION 4 and again integrating gives:

$$R_1 = \frac{(B_o - B_t)\ln\frac{i_o}{i_t}}{t\ln\frac{B_o}{B_t}} \tag{4b}$$

$$R_2 = R_1 + \frac{B_o - B_t}{t} \tag{5b}$$

B_{t}

cal with the differential equations used by these authors in evaluating from a

nflow and outflow (before and

which injected glucose mixes

, namely:

$$R_A = -\frac{N}{\gamma}\frac{d\gamma}{dt} \tag{4c}$$

$$R_D = R_A - \frac{dN}{dt} \tag{5c}$$

When F is not zero, as in the priming dose-constant infusion experiments, integration of EQUATIONS 4 and 5 gives the expressions originally employed in our own work[5] except for the differences in the symbols employed, that is, for EQUATION 4

$$\frac{i_t - \frac{F}{R_1}}{i_o - \frac{F}{R_1}} = \left(\frac{B_o}{B_t}\right)^{\frac{R_1 t}{B_t - B_o}} \tag{4d}$$

or when R_1 is zero (endogenous inflow stops):

$$i_t = i_o + \frac{Ft}{B_t - B_o} \ln \frac{B_t}{B_o} \tag{4e}$$

and for EQUATION 5

$$R_2 = R_1 + \frac{B_o - B_t}{t} \tag{5}$$

EQUATION (4e) is used as an identity that must be satisfied if R_1 is to be zero, whereas if R_1 is not zero equation (4d) gives its value

It is seen from the above that in those experiments with C^{14} glucose in which calculations have been made of glucose production (R_1) and utilization (R_2) after insulin injection that the same basic model and assumptions have been adopted by all investigators However, all of them have not used the same method for determining the size of the glucose pool to be used in calculation

Dunn *et al*[3] have adopted the usual tacit assumption that intravenously injected glucose mixes rapidly with the whole of the glucose pool On this basis they calculate pool size by extrapolating the curve of diminishing blood glucose-specific activity back to the time at which a single injection of C^{14} glucose of known total activity was given intravenously Subsequent to the injection of insulin they use the whole of this pool in their calculations Wrenshall *et al.*[4] do the same thing, although they speak of using a "central compartment" in which injected glucose mixes rapidly relative to its rate of inflow and outflow In our own work[5] we have calculated the magnitude of the error in evaluating pool size that is incurred by extrapolating back to zero time the specific activity values observed between 60 and 180 min on the assumption that these observed values refer to a well-mixed pool We have demonstrated

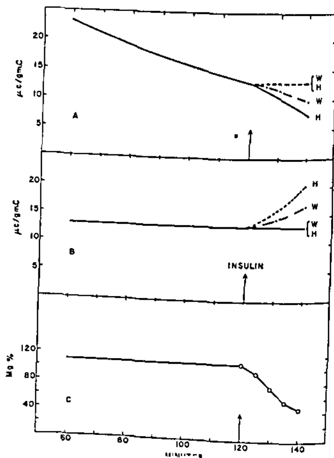
determined in this way by the "priming dose-constant infusion" technique because only about one half of the pool responds rapidly to changes in the rate at which C^{12} glucose enters from the liver

The Influence of Injected Insulin

are similar The same proportionate difference in observed specific activity is predicted for either technique when a reduction occurs in the rate of C^{12}-glucose output by the liver It should be noted that in calculating the expected

into cells of the glucose in the slowly mixing half of the pool, this higher spe-

cific-activity glucose should as time goes on mix with the fast half of the po and raise the observed specific activity above the curves shown in panel · It was felt to be more suitable for the present purpose of comparing the sens tivities of the two methods to accept the tenet under which the single injectic calculations have been made, that is, instantaneous mixing throughout tl glucose pool, whatever value may be taken for its size. However, it shoul



be noted that the magnitude of the change that would be brought about by the entry of higher specific-activity glucose from the slow half of the pool is

for which there is no experimental evidence, these thoughts were not included

instantaneous mixing is assumed to occur in the whole glucose pool, Dunn and

on the basis of whole-pool rather than half-pool size, they would represent a somewhat larger influence of insulin on hepatic glucose output than was reported

However, this difference in emphasis brought about by the methods of calculation does not represent the entire difference between the results by the two methods of using C^{14} glucose In this connection it should be noted that the single-injection procedure, under somewhat different physiological conditions, does not always give the above result Thus, Tarding and Schambye[7] and we,† using anesthetized normal dogs, have not obtained the long plateau in

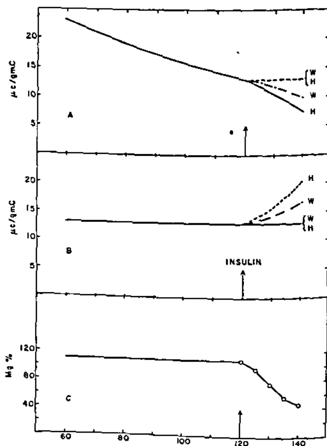
unanesthetized normal rabbits, at first reported[8] findings indicating cessation of

in the hepatic vein blood *During the period of falling blood glucose concentration they observed a gentler fall in hepatic vein blood glucose-specific ac-*

* This possibility was expressed to me by G I Searle and M S Goldstein in the spring of 1958.

† Unpublished experiments performed in collaboration with J S Bishop and R Levine

tivities of the two methods to accept the tenet under which the single injection calculations have been made, that is, instantaneous mixing throughout the glucose pool, whatever value may be taken for its size. However, it sho



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μc C^{14}/min continuously

The Influence of Endogenous Insulin Evoked by Injection of Glucose

It has been mentioned above that in calculation from the plasma glucose-specific activities and concentrations after insulin we have adopted the use of ... to 180- ... beginning ... after the initial priming injection of C^{14} glucose is that prior to that time the complete mixing of the injected glucose with the whole of the body glucose pool has not been approached closely enough. This phenomenon has been submitted[8] to mathematical analysis, using a model of the body glucose pool consisting of a central plasma compartment and two peripheral tissue fluid compartments.

On this basis it was concluded that one of the compartments, the glucose of which, together with the glucose of plasma, makes up about one half the total pool, mixes quite rapidly with plasma glucose, whereas the other peripheral compartment mixes slowly.

continues to produce C^{12} glucose so as to balance the C^{14} glucose of higher specific activity entering during equilibration from the slower mixing part of the glucose pool. An obvious way to test this was to give a glucose load tagged

cient to maintain a steady plasma glucose concentration. A typical result, obtained in each of four such experiments carried out in collaboration with

with the idea that there is a slowly mixing part of the body glucose pool as concluded earlier[8] from our experience with injected C^{14} glucose.

plished. Consequently in FIGURE 4, in which the sensitivities of the two tech-

in rate of C^{12}-glucose production by liver is more difficult to detect than in the insulin experiments because the glucose pool whose specific activity must be

tivity (the "plateau" effect), but also found that during this period the specific activity of the portal vein blood showed an even more marked deviation from its preinsulin course Since in their experiment there was no infusion of C[14] glucose after the first single injection, such an effect on portal glucose-specific

et al was that insulin facilitates re-entry into the blood of glucose sequestered by the cells of the intestinal mucosa. For the model experiment shown in FIGURE 2A it can be calculated* that the amount of sequestered glucose required to be returned to the circulating blood to give the 20-min. plateau after insulin on the assumption that hepatic C[12]-glucose output continues undiminished is at least

that the group associated with Weinhouse also obtained an accentuated apparent action of insulin on hepatic glucose output[12, 13] that in their experiments seemed to be sufficient to account for the whole of the blood sugar-reducing action, leaving essentially no residue for peripheral action of insulin Thus our own results using the priming dose-continuous infusion technique are in this respect similar to those obtained with the single-injection techniques, but consistently indicate a lesser apparent effect of insulin on hepatic glucose output.

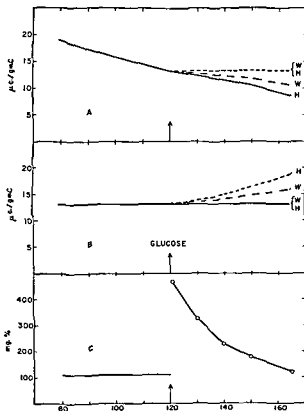
... initial and the 2-hour specific activities is only about one third as much as in the single-injection procedure (see FIGURE 2 and its legend, which gives the amounts of C[14] injected initially in the two cases).

In summary, the factors contributing to the changes in specific activity after insulin injection that seem to indicate an immediate diminution of hepatic glucose output are not yet fully understood. Further elucidation of these factors is required before the C[14]-glucose technique can be stated to provide positive evidence for an action of insulin to inhibit hepatic glucose output

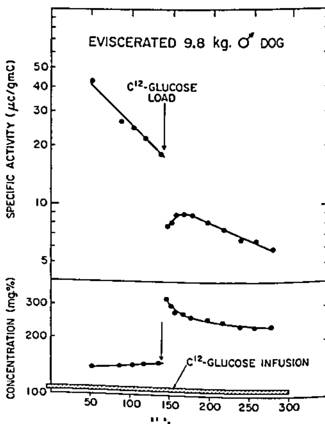
* The making of this calculation was suggested by conversations with Arnold Dunn

ment demonstrates that one should expect a lack of action of injected insulin on hepatic glucose output The reason for this is that, although there is very

insulin preparations.



affected is larger, but again it is seen that there is no difference between the two methods as regards their sensitivity for detection of such a change. Furthermore, since the actual experiment using the priming dose-continuous infusion technique results in very little change in specific activity, the importance of



[illegible] constant infusion was at 120 mg/kg/hr Reproduced by permission from *The American Journal of Physiology*

whether the whole-pool size or the half-pool size is used in calculation is mini-

[illegible] glucose output. How-
there is some doubt about the converse conclusion, namely, that this experi-

EFFECTS OF EXOGENOUS AND ENDOGENOUS INSULIN ON GLUCOSE UTILIZATION AND PRODUCTION*

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Department of Pharmacology, New York University College of Medicine, New York, N Y, and The Department of Biology, Brookhaven National Laboratory, Upton, N Y

Recent findings reported in the literature concerning the mechanism of insulin action are in some respects contradictory The observations originally made by Gemmill[1-3] that the addition of insulin to the incubation medium enhances the glucose uptake and glycogen formation by the isolated diaphragm

(L L. Madison, B Combes, W Strickland, R Unger, and R Adams, Unpublished data) It was postulated further that endogenous insulin secreted under physiological conditions exerts a similar effect on hepatic glucose release[4]

The studies reported here represent a continuation of earlier work[7] undertaken to determine whether insulin exerts its action on the peripheral tissues

and without any operative procedure, under truly physiological conditions, using C^{14} glucose

Preliminary Considerations

Summary

Differences in the observed results as well as differences in interpretation of results of the various C^{14}-glucose methods seem to be responsible for the differ

It is probable that differences in the physiological states of the experimental animals are involved in other instances of varying results

The weight of the evidence bears out the view that inhibition of hepatic glucose output is not the most important factor in the immediate blood sugar

insulin actually secreted renders this an unsatisfactory indication that one should expect a complete absence of any direct hepatic inhibitory effect of

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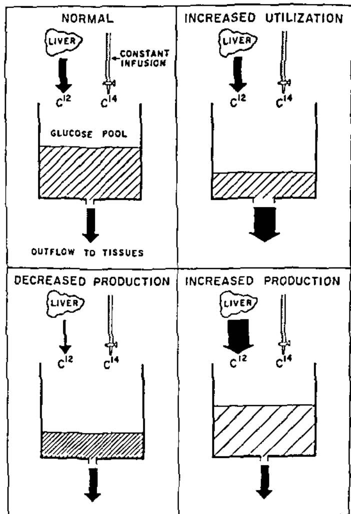
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NORMAL
LIVER
CONSTANT INFUSION
C^{12}
C^{14}
GLUCOSE POOL
OUTFLOW TO TISSUES
INCREASED UTILIZATION
LIVER
C^{12}
C^{14}
DECREASED PRODUCTION
LIVER
C^{12}
C^{14}
INCREASED PRODUCTION
LIVER
C^{12}
C^{14}

as the amount of glucose that dilutes the administered C^{14} glucose, the turnover rate of the glucose pool as the rate of removal of plasma glucose by the tissues (glucose utilization) and the rate of replacement of plasma glucose (glucose production by the liver) In the resting postabsorptive state, when the plasma-glucose concentration remains constant and no glucose is excreted in the urine, glucose utilization and production are equal

In the experiments reported here, the body glucose pool was labeled by an intravenous priming injection of a trace amount of uniformly labeled C^{14} glucose. The established radioactivity of the pool was then maintained by a

the size and turnover rate of the body glucose pool were determined

In a number of experiments insulin was injected during the course of constant infusion of C^{14} glucose [7] If the resulting decrease in the size of the body glucose pool and plasma-glucose concentration were due to an increased glucose utilization by the tissues without affecting the release of glucose from the liver, the specific activity would remain unchanged during the fall of the plasma-glucose concentration (FIGURE 1, *upper right*). Since in this case the glucose inflow from the liver does not change, the C^{14} glucose infused by constant infusion still balances the endogenous C^{12} glucose released by the liver If, however, the fall in plasma-glucose concentration were due to a decrease in glucose production, then the infused C^{14} glucose would no longer be balanced by the endogenous C^{12} glucose inflow from the liver, and the specific activity of the plasma glucose would rise during the period of development of the hypoglycemia (FIGURE 1, *lower left*) A fall in specific activity indicates that there is an increased C^{12} glucose inflow from the liver (FIGURE 1, *lower right*) This is shown very markedly when the plasma-glucose concentration returns from the insulin-induced hypoglycemic level to its preinsulin level, signifying that this process is due to an increased glucose release from the liver.

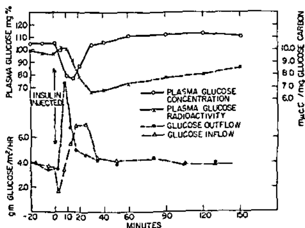
The method just described, consisting of priming injection followed b constant infusion of a trace amount of C^{14} glucose, gives more accurate result for glucose pool size and turnover rate in the preinsulin period than either th single-injection technique or a technique involving continuous infusion with out a priming dose In a critical analysis[8] the magnitude of the errors incurre in using these various procedures of administering the C^{14} glucose was evaluated During the period following the administration of either insulin or a gluco load, the only circumstance under which perfectly accurate calculated gluco inflows [illegible] that, first, the whol glucose [illegible] y, and second, that th plasma-[illegible] time. In the period o falling [illegible] are better satisfied, by the continuous infusion techniques than by the single-injection procedure When recovery from hypoglycemia (in the case of the insulin experiments) takes place and the plasma-glucose specific activity does fall markedly as a result of increased hepatic glucose output, our practice of using the half-pool rather than the whole-pool size in making calculations of glucose inflow minimizes the

derivative was converted to the glucosotriazole derivative, and the glucose-specific activity was determined by the liquid scintillation counting method of Steele *et al.*[15] The method for calculation of the glucose pool size and the rates of plasma glucose production and utilization in the steady state[6] and during periods of changing plasma-glucose concentration[7] are given elsewhere

Results

Effect of Insulin Administered by a Single Injection into a Peripheral Vein of the Normal Dog

After a 2-hour preliminary period, during which C^{14} glucose was administered as described above to determine the control size and turnover rate of the



glucose pool, insulin (low in glucagon content) was administered as a single injection into either the saphenous or femoral vein FIGURE 2 shows the data

[illegible]

of the plasma-glucose specific activity The calculated rate of glucose uptake by the tissues shows a marked increase, while the calculated rate of glucose

errors caused by the fact that new glucose introduced into the blood mixes rapidly with only about one half of the total glucose pool.[7] With regard to recycling, by which is meant the reincorporation into glucose of fragments containing tagged glucose carbon removed earlier from the blood by the tissues, the continuous-infusion methods minimize the disturbing effect of this phenomenon, inasmuch as the returning tagged fragments are entering a pool maintained at a higher level of specific activity than in the single-injection

experiment will have a lesser disturbing effect on calculated inflow in continuous-infusion experiments than in single-injection experiments.

Materials and Methods

The experiments were performed on trained adult normal and hypophy-

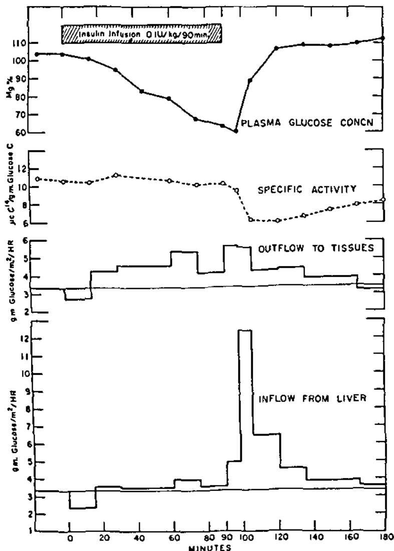
into the external jugular vein of the same C^{14} glucose at a rate that matched the inflow rate of C^{12} glucose from the liver into the plasma, provided the inflow remained unchanged[8] The C^{14}-glucose infusion was also continued uninter-

of C^{14} glucose continued throughout the entire experiment.

In the
glucose
infusion
glucose.
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At various intervals samples of blood were drawn from an exposed femoral vein, delivered into heparinized tubes and centrifuged, and the plasma immediately separated. The plasma-glucose concentration of each sample was determined, using aliquots of Somogyi zinc-barium filtrate[11] by two methods those of Hagedorn and Jensen[12] and Nelson[13] A sample of glucose from each filtrate was isolated as the phenylosazone derivative and the glucose specific activity determined by either of two methods In earlier experiments, the derivative was burned to CO_2 by wet combustion, and the $C^{14}O_2$ counted by the method of Van Slyke *et al*[14] In later experiments, the phenylosazone

* We are indebted to O K Behrens, Eli Lilly and Company, Indianapolis, Ind. for his donation of the insulin



Insulin Infusion 0.1U/kg/90min.
Mg %
110
100
90
80
70
60
PLASMA GLUCOSE CONCN
μc C14/g m. Glucose C
12
10
8
6
SPECIFIC ACTIVITY
g m Glucose/m2/HR
6
5
4
3
2
OUTFLOW TO TISSUES
g m. Glucose/m2/HR
12
11
10
9
8
7
6
5
4
3
2
1
INFLOW FROM LIVER
0
20
40
60
80
90
100
120
140
160
180
MINUTES

It can also be seen in FIGURE 2 that during the recovery period the return of the plasma glucose concentration to its preinsulin level is accompanied by a considerable decrease in the plasma-glucose specific activity. The glucose production by the liver is greatly increased during this period and, since the glucose utilization does not decrease below the preinsulin level, the return of the plasma-glucose concentration to control level is due entirely to the increased glucose production

With larger doses of insulin the resulting hypoglycemia was more marked, and the glucose utilization was increased to a greater extent and for a longer period than following smaller doses of insulin. The rate of enhancement of

the increased glucose release by the liver was evoked. Thus, there did not appear to be a critical level of hypoglycemia at which the liver started to release increased amounts of glucose

Effect of Insulin Administered by a Prolonged Constant Infusion into a Peripheral Vein of the Normal Dog

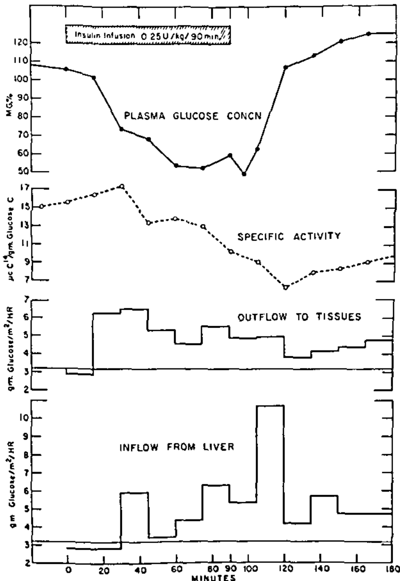
tion fell during the insulin infusion and that the hypoglycemia persisted throughout the period of insulin infusion The corresponding plasma-glucose specific activity remained remarkably uniform at the preinsulin level. The rate of glucose uptake by the tissues was greatly increased during the insulin

by the liver.

Upon the termination of the insulin infusion the plasma gl... concentration

comp

o the preinsulin value since
ontrol value and indeed was



The glucose inflow increased significantly shortly after termination of the insulin infusion and the plasma glucose concentration was rapidly elevated. Reproduced by permission from [illegible]

FIGURES 4 and 5 show the results of 2 experiments in both of which 0.25 U./kg. of insulin was infused intravenously in 90 min. As expected, in both cases the plasma-glucose concentrations fell more rapidly and to lower levels than in FIGURE 3, where a smaller dose of insulin (0.1 U./kg.) was used. The plasma-glucose concentrations remained at the low levels during the insulin infusions. In both experiments during the development of hypoglycemia there was a transient rise in the plasma-glucose specific activity. The rates of glucose utilization were markedly increased. The rates of glucose production shortly after the start of insulin infusion decreased, to a greater extent in the experiment shown in FIGURE 5 than in that shown in FIGURE 4, indicating that, at least in these experiments, the initial fall in plasma-glucose concentration was due to both an increased glucose utilization and diminished glucose production.

During the course of the insulin infusion, when the plasma-glucose concentrations remained at the low levels, the glucose utilization continued at increased rates. At the same time the rates of glucose production were also increased, but not adequately enough to elevate the plasma-glucose concentrations.

As in FIGURE 3, in these experiments the termination of insulin infusion was followed by a rapid rise of the plasma-glucose concentration to control levels, and this was accompanied by a considerable reduction of the plasma-glucose specific activity values. The [illegible]
4 [illegible]
th [illegible]

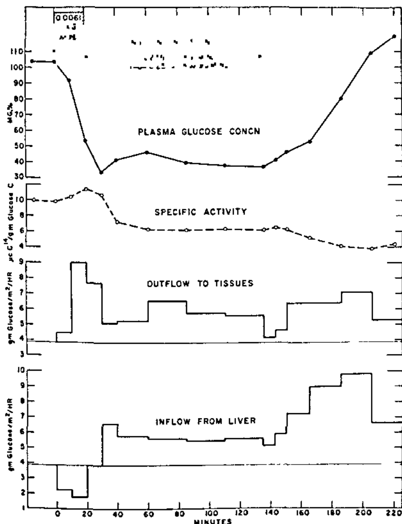
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[illegible] by the liver.

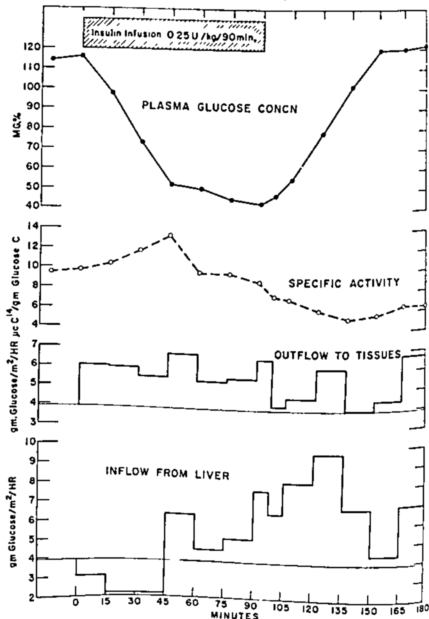
After the initial 20 min the rate of insulin infusion was reduced to 0.0028 U./kg./m [illegible] shown in FIGURES 4 and 5 [illegible] infusion was maintained fo [illegible] 15 min, in addition to the higher rate during the initial 20 min.

The marked increase in glucose production appeared as in previous experiments only after the insulin infusion had been terminated (135 min.) and again was responsible for the return of the plasma-glucose concentration to control levels.

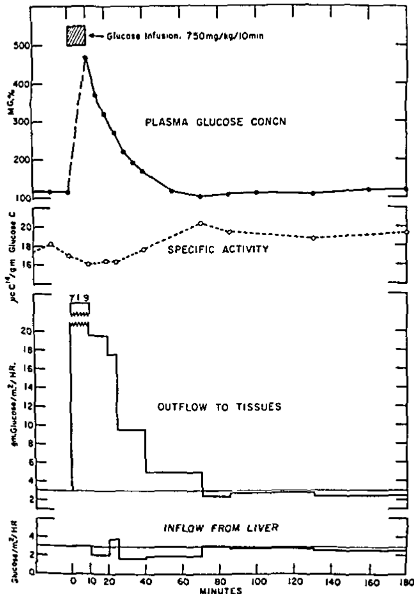
In one dog, insulin was injected subcutaneously (0.15 U./kg.). The hypoglycemia developed slowly, reached the lowest concentration at 80 min and continued at this level until the termination of the experiment at 120 min. The attending changes in glucose utilization and production were similar to those observed when insulin was administered by intravenous infusion. Thus,



but was markedly increased following cessation of insulin infusion and elevated the plasma-glucose levels.



Insulin Infusion 0.25 U/kg/90 min.
PLASMA GLUCOSE CONCN
MG%
SPECIFIC ACTIVITY
μc C[14]/gm Glucose C
OUTFLOW TO TISSUES
gm. Glucose/m[2]/HR
INFLOW FROM LIVER
gm Glucose/m[2]/HR
MINUTES



of plasma glucose to the tissues." The glucose inflow from liver was slightly reduced. Reproduced by permission from *Metabolism*.[16]

Effect of a Glucose Load Administered into a Peripheral Vein of the Normal Dog

Endogenous insulin released under physiological conditions traverses the liver before reaching the peripheral circulation. In view of this, it was desirable to compare the effects on glucose production and utilization observed

during the period of hyperglycemia. It is readily apparent from FIGURE 7 that the return of the plasma-glucose concentration from the hyperglycemia to control levels was accomplished entirely by an increased removal of the glucose from the plasma. Some of this glucose was excreted in the urine, but this accounted for only 8 per cent of the glucose load.

In other experiments glycosuria was avoided by administering the glucose load over a prolonged period. In the experiment shown in FIGURE 8, the glu-
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that the c
level was ... by the tissues.

evokes the secretion of IGURES 7 and 8 that the tilization and production are similar to the effects produced by administration of ins

Effect of Insulin Administered by a Single Injection into a Peripheral Vein of the Hypophysectomized Dog

The preceding observations show that during the course of infusion of insulin in the normal dog there is a limitation in the increase in glucose production in

of insulin infusion

5, and 6), although

A more marked

increase in glucose production occurs following termination of insulin infusion

Earlier studies have shown that several hormones influence glucose production[4] In order to throw light on the interplay of the various hormones on glucose production, the effects of insulin on glucose production and utilization were studied in hypophysectomized dogs The hypophysectomized animal is extremely sensitive to the action of insulin, as evidenced by the observation that injection of small doses of insulin results in a severe and prolonged hypo-

the normal animal (FIGURE 2) and explains the greatly delayed return of the plasma-glucose concentration following insulin injection to the control level in the hypophysectomized dog Since the well-nourished hypophysectomized

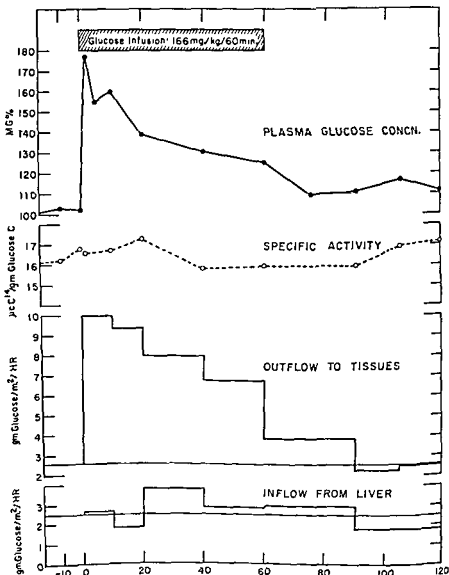
DISCUSSION

From the data presented in this paper it is evident that in every experiment, regardless of whether insulin was administered by a single intravenous injection, by continuous intravenous infusion, or by subcutaneous injection, the rate of glucose utilization was consistently increased during the development

lowered as a result of an increased utilization of glucose Thus, it may be

during the insulin infusion, despite the severe hypoglycemia, the glucose produc-

er stayed at the preinsulin level or, if it did increase above the pre-


Glucose Infusion: 166mg/kg/60min
MG%
PLASMA GLUCOSE CONCN.
μc C14/gm Glucose C
SPECIFIC ACTIVITY
gm Glucose/m2/HR
OUTFLOW TO TISSUES
gm Glucose/m2/HR
INFLOW FROM LIVER
-10 0 20 40 60 80 100 120

Effect of Insulin Administered by a Single Injection into a Peripheral Vein of the Hypophysectomized Dog

The preceding observations show that during the course of infusion of insulin in the normal dog there is a limitation in the increase in glucose production in

of the hypoglycemia.[21] As can be seen in FIGURE 9, the glucose production in the hypophysectomized dog did not increase despite the severe hypoglycemia produced by insulin. This is in marked contrast to the changes occurring in the normal animal (FIGURE 2) and explains the greatly delayed return of the

DISCUSSION

From the data presented in this paper it is evident that in every experiment, regardless of whether insulin was administered by a single intravenous injection, by continuous intravenous infusion, or by subcutaneous injection, the

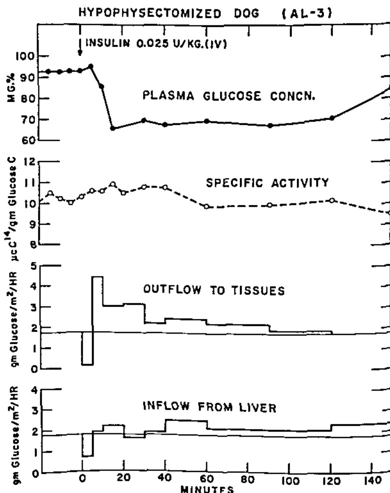
The effects of endogenous insulin, the secretion of which was evoked by the administration of an intravenous glucose load, are also in complete harmony

glucose by the tissues.

Of the changes observed in glucose production during and following insulin administration, the most outstanding and consistent observation was the "*restraining effect*" of insulin on the *increase in glucose production*. This effect was noted in every experiment and manifested itself in the following way: during the insulin infusion, despite the severe hypoglycemia, the glucose production either stayed at the preinsulin level or, if it did increase above the pre-

insulin level, the increase was not sufficient to elevate the plasma-gl concentration to control levels A marked and adequate increase in gl production occurred only after the insulin infusion was terminated, at time the enhanced glucose production restored the plasma-glucose conce tion to control levels

Whether the restraining effect of insulin is a direct action of insulin o liver or is brought about indirectly, or whether insulin interferes with



glucose levels

action of the blood sugar-raising hormones cannot be established at present. Recent unpublished experiments with phlorhizin have shed some light on this problem The administration of phlorhizin to dogs in which the glucose pool was labeled as described above resulted in excretion of glucose in amounts

of the animal The

plasma-glucose con-

while both phlorhizin

and insulin bring about an increased removal of glucose from the plasma, the glucose production is restrained only in the case of insulin It would appear then that insulin or changes attended with the increased glucose utilization brought about by insulin are instrumental in the restraint of glucose production.

As has been shown, the ability to release glucose in response to hypoglycemia is influenced by various hormones [21, 22, 25] This is evident from the observations that, in the hypophysectomized (FIGURE 9) or adrenalectomized dog,[23] the increase in glucose production following insulin-induced hypoglycemia

nals were

[22, 26] and

or 11-17

oxycorticosteroids[24] to the hypophysectomized dog restores the ability to increase glucose production in response to insulin-induced hypoglycemia In addition, the steroids,[24] but not growth hormone (unpublished data), restore the sensitivity of the animal to the hyperglycemic action of adrenaline or glucagon Thus, it is conceivable that the "restraining action" of insulin on glucose production may involve interference with the secretion or action of these blood sugar-raising hormones

In some experiments immediately following the injection of insulin or at the beginning of the infusion of insulin, a transient rise in specific activity was noted. This effect was not present in every experiment and, when it did occur, its magnitude showed considerable variations. As stated above, this observation would indicate a decrease in glucose production that would be a contributing factor, in these experiments, to the development of the insulin hypoglycemia However, in view of some recent findings of Shoemaker *et al*[27] and of Mahler

Summary and Conclusions

The effects of exogenous and endogenous insulin on glucose production an utilization were studied in unanesthetized normal and hypophysectomized dog using C^{14} glucose

Insulin, administered intravenously by single injection or continuous in fusion, consistently increased the utilization of plasma glucose. The increase glucose utilization observed in the course of the insulin infusion was evider throughout the period of development and maintenance of the hypoglycemia as well as during the return of the plasma glucose to control levels.

Similarly, plasma-glucose utilization was increased as a result of the intra venous administration of a glucose load, which presumably provoked th secretion of insulin

The restoration of the plasma glucose concentration from the insulin-induce hypoglycemia to the preinsulin levels was invariably due to an increase i glucose inflow from the liver into the plasma. During the infusion of insuli an increase in glucose production was observed in some experiments, but was of a limited nature and did not restore the plasma-glucose concentratio to control levels Upon termination of the insulin infusion the glucose inflo increased markedly and rapidly restored the control plasma-glucose level It thus appears that the increased glucose production in response to hypogly

:tion was observed soo

, and contributed to th

development of hypoglycemia

The deficient response of the hypophysectomized dog to the insulin-induce hypoglycemia throws some light on the involvement of the pituitary an adrenal gland hormones in this phenomenon

Acknowledgment

The authors gratefully acknowledge the technical assistance of G Durandy H R Bello, S Ambrus, and C. Bjerknes in these studies.

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The restoration of the plasma glucose concentration from the insulin-indu

cemia is restrained during the insulin infusion

In some experiments a diminished glucose production was observed s

Acknowledgment

The authors gratefully acknowledge the technical assistance of G. Duran H. R. Bello, S. Ambrus, and C. Bjerknes in these studies.

support this finding At 45 min. after the intravenous injection of tolbutamide (60 mg/kg) the glucose space was reduced to one third of its preinjection value in a normal dog

The mass of glucose with which labeled glucose added to the blood intermixes rapidly and its changes with time are essential for the calculation of rates of [illegible]

To sum up, a tracer-determined glucose space in the dog appears to be subject to marked changes following the intravenous injection of either insulin or tolbutamide Thus rates of glucose inflow and outflow, calculated on the assumption that this space remains constant after injection of these substances, are of uncertain reliability

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to remain constant thereafter where estimations of glucose inflow and outflo
rates were made

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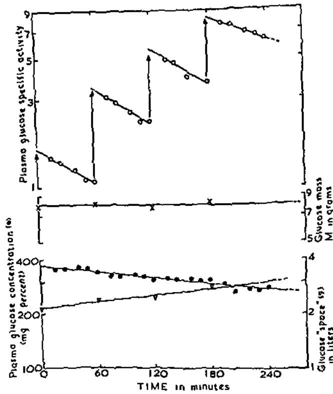


FIGURE 1 Blood plasma glucose concentration (C), glucose mass (M), and glucose space ($V = \frac{M}{C}$) in an anesthetized, depancreatized dog 48 to 52 hours after insulin The vertical arrows indicate the times of intravenous injection of C[14] glucose

dog by G H
1). These
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is more than doubled at 1 hour after the intravenous injection of insulin (1 U./kg). The increase in the glucose space of the isolated perfused rat heart caused by insulin, and reported in this monograph by C. R. Park, appears to

Krahl[16] has demonstrated that insulin will accelerate the synthesis of glutathione in liver slices from diabetic rats. Glucose must be present in the system in order to demonstrate an insulin effect. It was also observed that this effect could be obtained only within a few days after the injection of alloxan.

Since the liver can produce as well as utilize glucose, it is difficult to demonstrate a net glucose uptake by this tissue. However, a decreased splanchnic-glucose output has been observed following insulin administration.[17,18] Assuming that the nonhepatic contribution to the metabolism of splanchnic area ... sulin to ... Using ... per cent glucose, Haft and Miller[19] found that the glucose concentration of the perfusing medium increased to 600 mg. per cent within 1 hour of perfusion, and that this increase was unaffected by insulin. However, with livers from alloxan-diabetic rats, insulin produced an increased removal of glucose at these concentrations and after 1½ to 4 hours of perfusion. It was noted further that liver of ketotic animals did not respond.

In any consideration of a hepatic action of insulin one must take into ac- ... formed with the intact animal, one must also take into account the fact that any rapid fall in blood glucose induced by insulin may cause increased hepatic glycogenolysis. Whether this is due to a release of endogenous glucagon or epinephrine is not clear, however, the possibility does exist that such factors might be released and would tend to override any direct effect of insulin on hepatic tissue. These and other factors that might be involved in attempts to demonstrate a hepatic action of insulin have been more extensively reviewed by deDuve[20] and by Levine and Fritz.[21]

In Vivo Studies

... by the fact that under ... The inevitable result is ... n attempt to circumvent ... f different methods have ... area, with obvious contributions of extrahepatic tissue. Following insulin administration, a decrease in the splanchnic glucose gradient has been observed, however, it is not possible to attribute such effects to the action of insulin on hepatic tissue.

Hepatic portal venous glucose gradients. The hepatic contribution to splanchnic-glucose output can be derived from measurements of glucose concentrations in portal venous and hepatic venous blood. Glucose gradients across the liver have been reported by Ashmore *et al.*,[22] using dogs with indwelling plastic

HEPATIC ACTION OF INSULIN

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James Ashmore
Department of Biochemistry, Indiana University, Indianapolis, Ind.

The role of insulin in regulating glucose metabolism by extrahepatic tissue is well recognized It is generally accepted that the action of insulin on

with both normal and diabetic preparations. With respect to the liver, however, there is at the present time considerable doubt as to the exact mechanism of insulin action on this tissue In contrast to muscle, liver cells are freely permeated by glucose,[9] therefore, any action of insulin to facilitate transfer of glucose must be upon some intracellular compartment. It has also been found that, while muscle from diabetic animals responds immediately to the addition of insulin, the diabetic liver is not restored to normal glucose metabolism upon the immediate administration of insulin [10,11] Treatment of the animal with insulin over a period of several days is required to return glucose metabolism of the diabetic liver to normal

In Vitro Studies

Chernick and Chaikoff[12] have studied the metabolism of C^{14} glucose by liver slices from normal and alloxan-diabetic rats and have concluded from their data that the impairment in glucose metabolism in the diabetic animal is due in part to an inability to phosphorylate glucose. Utilizing a similar technique, Renold *et al* [11] were able to devise a means of calculating glucose phosphorylation by liver tissue *in vitro* It was found that glucose phosphorylation by hepatic tissue from diabetic rats was only one fourth to one sixth as much glucose as tissue from normal rats It was subsequently shown[11] that 12 to 24 hours of insulin action *in vivo* were required to restore glucose phosphorylation of the diabetic liver to normal

These observations would lead one to believe that perhaps insulin has no direct action on the liver However, there have been a number of instances in

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sl... ... of glucose into the liver glycogen It has also been demonstrated that the addition of insulin to hepatic tissue *in vitro* will accelerate the synthesis of long-chain fatty acids [14,15] Increased lipogenesis and glycogen synthesis have been demonstrated only with tissue from normal animals, and neither effect has been uniformly reproducible

travenous injection of a tracer dose of highly active labeled glucose, the dilution of the plasma glucose can be estimated from the exponential decay of

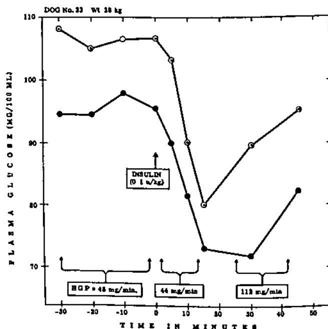
the specific activity of the plasma glucose This effect has been attributed to a direct action of insulin on the liver and has been interpreted to mean that, during the observed plateau, hepatic glucose production has completely stopped Under essentially the same conditions, except that an anesthetized dog was

insulin is not as simple as it might at first appear Measurements of specific activity and concentration of glucose in portal and hepatic venous blood clearly

dog was 118 mg /min Calculation of hepatic glucose production from portal-hepatic glucose gradients and plasma flow during the same period gives values

Lipscomb and Crandall[33] have used the London cannula to sample portal and hepatic blood in dogs From their studies they have calculated a mean hepatic

catheters. No decrease in the portal-hepatic glucose gradient was found in such animals following injection of insulin. In an extension of these studies by Shoemaker *et al*,[24] hepatic plasma flow was estimated by a modification of the Bromsulphalein clearance method of Bradley.[25,26] Plasma-glucose concentrations for a typical dog studied are shown in FIGURE 1. Hepatic glucose production calculated from portal-hepatic glucose gradients and plasma flow for the preinsulin period was 48 mg./min. No change was observed during the 10 min immediately following insulin; however, a sharp increase in hepatic glucose production was observed with the onset of hypoglycemia.



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2.1 ± 0 65 (S.E.) mg /min. The hepatic-glucose output during the control period was 2 0 ± 0 33 mg./kg /min, and this increased to a mean of 3.9 ± 0 39 (S E) mg /kg /min for the postinsulin period.[24] Although an effect of insulin to decrease splanchnic glucose production was observed, no evidence of a decrease in hepatic glucose production was obtained.

Estimation of hepatic glucose production by isotope dilution. Following in-

tory.[27, 28] Henderson *et al*.,[31] using the isotope dilution method in diabetic dogs, have calculated glucose production to be of the order of 0 29 gm /kg /hr

Steele *et al.*[33] have measured glucose production in dogs by isotope dilution, but have employed the technique of constant infusion of the labeled glucose Under these conditions a mean glucose production of 0 15 gm /kg /hr has been obtained from 10 experiments on 7 dogs

Tarding and Schambye have also reported determinations of hepatic glucose production, using both the isotope-dilution and hepatic-catheterization techniques[30] The values obtained by these authors are summarized in TABLE 2, the values from our own series are in parentheses The data of Tarding and Schambye are reported in mg /min , and our own values have been computed on the same basis for comparison In both the preinsulin and postinsulin con-

case

TABLE 2
THE EFFECTS OF INSULIN ON HEPATIC GLUCOSE PRODUCTION IN DOGS*

	Method used to estimate hepatic glucose production	
	Isotope dilution	Liver catheterization
Preinsulin	80 mg /min (118 mg /min)	30 mg /min (44 mg /min)
Postinsulin	60 mg /min	40 mg /min (61 mg /min)

* From the data of Tarding and Schambye[30]

an exchange could be mediated by glucose-6-phosphatase or amylo-1, 6-glucosidase[34]

Conclusions

There is little doubt that insulin has an important role in the regulation of liver metabolism since, in the absence of the hormone, profound changes in hepatic metabolism occur Indeed, many of the difficulties in the treatment

Under certain conditions it is possible to demonstrate effects of insulin on hepatic metabolism However, the circumstances under which such effects

glucose production of 0 121 ± 012 (S.E.) gm /kg./hr. This result agrees with values that we obtained using the liver-catheterization technique. Using the isotope-dilution method, Dunn *et al.*[29] report values for hepatic glucose production in dogs ranging from 0 12 to 0 30 gm./kg /hr., which again would tend to be slightly higher than that observed by hepatic catheterization. Values ranging from 0 18 to 0 29 gm /kg /hr have been reported from Chaikoff's labora-

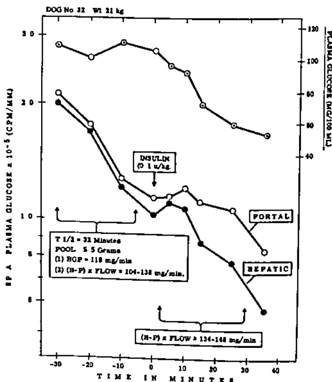


FIGURE 2 Estimation of hepatic glucose production by isotope dilution. Plasma-glucose concentration in mg /100 ml is given in the upper curve. Specific activity of portal and hepatic plasma glucose is plotted in lower curves

TABLE 1
ESTIMATION OF HEPATIC GLUCOSE PRODUCTION IN DOGS

Isotope dilution gm /kg /hr	Liver catheterization gm /kg /hr	Reference
0 35	0 16	Present study
	0 12	Lipscomb and Crandall[28]
(0.12 to 0 30)		Dunn *et al* [29]
0 34		Feller *et al* [27]
0 18		Searle and Chaikoff[28]
0 29*		Henderson *et al* [31]

* Diabetic dogs

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are obtained are such that at the present time no uniform theory of action of insulin on hepatic tissue can be formulated.

There is some question as to the validity of the estimation of hepatic glucose production by the isotope-dilution method. A plateau of plasma-glucose-specific activity after insulin administration cannot be attributed to an inhibition of hepatic glucose production in the light of direct measure of glucose production by hepatic catheterization.

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tissues to the liver. None of the available data on sulfonylurea hypoglycemia are inconsistent with this hypothesis, and many of the hitherto inexplicable actions of the sulfonylureas might be resolved if they were assumed to induce

support to the essential part played by the pancreas in arylsulfonylurea hypoglycemia and suggest that the major site of action of the increased insulin activity is on the liver The removal of the liver, however, results in an increased insulin action in the peripheral tissues

Intraportal and Peripheral Insulin on D-Xylose Disappearance

(FIGURE 1) Tolbutamide (25 mg/kg), however, had no such effect, which

blood glucose was measured.

Methods

intravenously into an arm vein over a 4-min period Twenty min later a blood sample was drawn from a separate forearm vein, and further samples

free) (0 1 U/kg) was injected over a 2-min period into the exposed portal vein. The same procedure was employed after the postoperative recovery

FURTHER STUDIES ON THE SIGNIFICANT ROLE OF THE LIVER IN SULFONYLUREA HYPOGLYCEMIA*

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The most disturbing inconsistency between sulfonylurea-induced and insulin-induced hypoglycemia has been the repeated failure to demonstrate that the sulfonylureas induce hypoglycemia by either a direct or insulin-mediated action on glucose metabolism in the peripheral tissues. There now appears to be little question that the presence of the pancreas is an essential prerequisite for the hypoglycemic action of a sulfonylurea,[1,2] and some studies indicate that part of the action of the sulfonylureas may depend on the presence of insulin[3-5] The degree of granulation of the beta cells appears to be related to the insulin content of the pancreas,[6,7] and the sulfonylureas have been shown to increase the quantity of islet tissue[8] and to decrease beta cell

support the insulinogenic action of the sulfonylureas. There are, however, only a few studies that actually show an increased insulin secretion when the insulin output is measured directly[14] The nature of the continuous or intermittent stimulation of the pancreatic beta cells remains unknown Other evidence has been cited to show that the sulfonylureas inhibit glucose-6-phosphatase activity,[16 17] and that they inhibit insulinase activity.[18] Other studies in depancreatized animals have demonstrated a hepatotoxic effect.[19 20] Some or all of these actions may contribute to the hypoglycemic action of the sulfonylureas in the absence of the pancreas. Nevertheless, the presence of the pancreas has proven vital to the action of the sulfonylureas; this being true, the absence of an increased peripheral "insulinlike action" following sulfonylurea administration remains a perplexing phenomenon[21]

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existence of a pancreatotropic action of tolbutamide, but merely transfers the emphasis on the major site of endogenous insulin action from the peripheral

* The work reported in this paper was supported in part by the John A Hartford Foundation, New York, N. Y , and The Upjohn Company, Kalamzaoo, Mich
† Research Fellow of the John A Hartford Foundation

Results In the 4 subjects studied postoperatively, insulin injected into a peripheral vein markedly increased the disappearance rate of the infused

[illegible], blood glucose fell [illegible] per cent after the peripheral vein injection of insulin and 50 per cent after the intraportal injection of

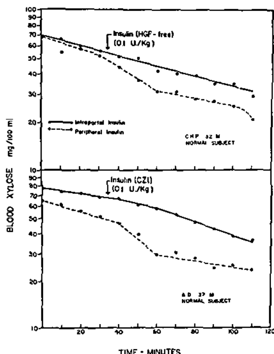


FIGURE 2 Intraportal and peripheral insulin action on pentoses Intraportally administered insulin had little or no effect on D-xylose disappearance Peripheral insulin consistently increased the disappearance rate of this pentose

also showed that operation alone had no effect on D-xylose disappearance (FIGURE 3). For comparison, this figure also shows the results obtained during

period in 4 of these patients, except that the insulin in the latter studies was injected into a peripheral vein Control studies were performed in 2 subjects ...e effect of operation alone on ... of operation on the D-xylose

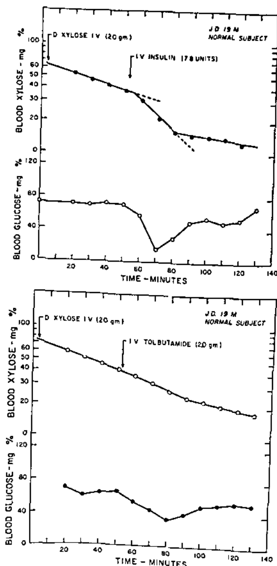


FIGURE 1 Insulin, but not tolbutamide, in man increased the disappearance rate of infused D xylose Both substances produced a marked hypoglycemia

the next study peripheral glucose uptake after tolbutamide administration was measured in dogs with porta caval shunts. In these animals, endogenous insulin is secreted directly into the general venous circulation, and tolbutamide administration in them would be expected to result in an increased peripheral insulinlike action similar to that seen following an intravenous insulin infusion or injection.

Methods. Four healthy mongrel dogs weighing from 20 to 40 lb were subjected to operation at which a side-to-side anastomosis was made between the portal vein and the inferior vena cava, and the portal vein ligatured between this anastomosis and the liver. Thus, the normal flow of endogenous insulin from the pancreas to the liver was diverted to the general systemic venous circulation. At least 2 weeks were allowed to elapse postoperatively before performing studies on these animals. Each experiment was preceded by an overnight fast and, on the following day, the dogs were anesthetized with sodium pentobarbital, and the femoral artery and vein of a hind limb were exposed on one side. At 60 or more min after the completion of these preliminaries simultaneous arterial and venous blood samples were withdrawn by needle and syringe from the exposed femoral artery and vein. Arterial and venous blood samples were taken in this manner at 10-min intervals for the next 20 minutes, and then tolbutamide 50 mg/kg in 20 cc of normal saline was injected into a separate peripheral vein over a 4-min period. Arterial and venous blood samples were then drawn at 5, 10, 20, 30, 45, 60, 90, and 120 min after the injection of tolbutamide. Identical experiments were performed in these same dogs, using crystalline zinc insulin (HGF-free, 0.2 U/kg) in place of tolbutamide. The insulin was administered in 20 cc normal saline over a 3-min period into either a peripheral vein or a mesenteric vein. In other experiments in these same dogs a constant infusion of the same insulin preparation 0.5 U/kg in 70 cc normal saline was administered over a 90 min period into either a peripheral vein or a mesenteric vein. A Harvard pump was used to maintain a constant infusion rate. During one experiment of each of the above procedures blood samples were also drawn for serum inorganic

below the venous values. FIGURE 5 compares the effects of tolbutamide, insulin infusion, or insulin injection on arterial and venous blood glucose. The changes in serum phosphate and blood pyruvic acid are also shown. The

operation with an intraportal injection of insulin and the results obtained with an intravenous injection of insulin after operation. The implied hepatic retention of insulin could be due to either a marked and immediate hepatic retention of a considerable portion of the intraportally injected insulin or to a temporary hepatic retention followed by a slow hepatic release of insulin in amounts insufficient profoundly to affect D-xylose disappearance. That the latter hypothesis is probably the operative one is suggested by the slight effect of tolbutamide or intraportally injected insulin on D-xylose disappearance that

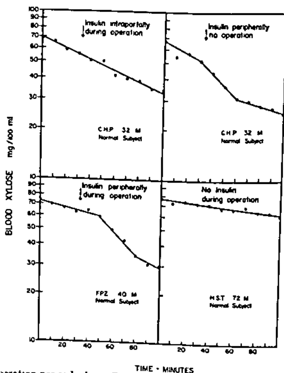


FIGURE 3. Operation per se had an effect neither on the disappearance of D xylose nor on the action of peripherally administered insulin.

was observed in some experiments and may be in part due to the sinusoidal character of the liver which, by the pooling of insulin in the liver, may result in a later, more gradual release of the unbound insulin.

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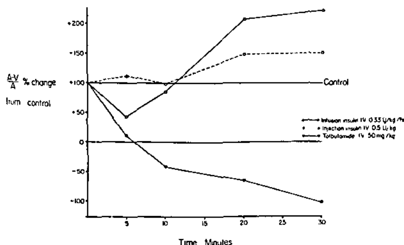
Tolbutamide Effects in Porta Caval-Shunt Dogs

These results led us to determine whether an initial transhepatic circulation of insulin could account completely for the differences previously observed between tolbutamide hypoglycemia and intravenous insulin hypoglycemia. In

effects of tolbutamide and insulin infusion on arterial and venous blood glucose concentrations are identical to those shown in FIGURE 4 The hypoglycemia accompanying insulin injection differed from that due to either tolbutamide or insulin infusion in that the arterial and venous glucose concentrations initially fell at the same rate, and no alteration of the usual arterial-venous glucose relationship occurred

The Effects of Tolbutamide on Peripheral Glucose Uptake $(A - V)/A$ in Porta Caval-Shunt Dogs

Peripheral glucose uptake can be expressed as the fraction of the arterial concentration that is removed by the limb, and this is represented by the



was found to contain this period of a rapid fall in glucose concentration, the changes in $(A - V)/A$ obtained during this period are shown in FIGURE 6

FIGURE 4 Peripheral glucose utilization in Eck fistula dogs The crosshatched area indicates there is a reversal of the normal arterial-venous glucose relationship for the first half hour after tolbutamide when compared with an insulin infusion.

FIGURE 5 Tolbutamide and insulin effects in Eck fistula dog, tolbutamide, insulin infusion, and insulin injection are compared Note the initially altered A-V glucose relationship after tolbutamide and the fall in pyruvic acid

Methods. Hepatectomized dogs received an intravenous injection of tolbutamide (50 or 100 mg/kg) during a glucose infusion administered into a jugular vein at a rate of 250 mg/kg/hour. This infusion rate was selected because these animals did not develop hypoglycemia at this rate and, by sup-

artery and vein by syringe and needle at 15-min intervals starting one half-hour after the completion of the hepatectomy Venous blood samples were

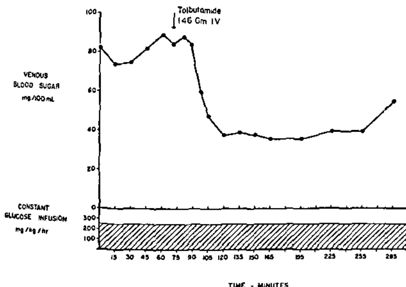


FIGURE 7. Effects of intravenous tolbutamide in blood sugar in the hepatectomized dog A prompt fall in blood sugar followed tolbutamide administration in the liverless dog

analyzed immediately for glucose concentration and, when a static blood glucose level had been present for 1 or more hours, tolbutamide (50 or 100 mg/kg in 20 ml of normal saline) was injected over a 4-min period into a peripheral vein. Simultaneous arterial and venous blood samples were then drawn at 15-min intervals for an additional 4 to 6 hours In 2 such experiments samples were also drawn from an indwelling catheter in a jugular vein for serum potassium and inorganic phosphate determinations

Results. The effect of intravenous tolbutamide (50 mg/kg) on the venous blood glucose concentrations of a hepatectomized dog is shown in FIGURE 7

This figure summarizes the results of all our studies on the effects of tolbutamide, insulin infusion, and insulin injection on peripheral glucose uptake in porta caval-shunt dogs The vertical axis represents the percentage increase or decrease in glucose uptake from the fasting control value, and any value below the 0 per cent line represents an arterial glucose concentration less than the venous glucose concentration. Intravenous sodium tolbutamide (50 mg /kg) resulted in a hypoglycemia and a progressive and regular decrease in the per cent uptake of glucose until the $(A - V)/A$ values became negative due to a drop in arterial glucose concentration to below the venous glucose concentration. Crystalline zinc insulin (HGF-free) 0.2 U./kg. injected into either a peripheral or a mesenteric vein in these porta caval-shunt dogs resulted initially in no significant change in glucose uptake despite a concomitant hypoglycemia This was soon followed by an increased glucose uptake Insulin (0 33 U /kg) administered by constant infusion into a peripheral or mesenteric vein resulted in an initial decrease in glucose uptake, later followed by an increased glucose uptake greater than that obtained with insulin injection, even though the total amount of insulin infused at the end of 30 min was still less than that given by injection

It has been frequently noted that decreased or negative $(A - V)/A$ differences following tolbutamide administration occur,[26-28] and no entirely satis-

resulting in an eventual lowering of the arterial glucose concentration. If the resulting rate of fall in arterial blood glucose concentration was in excess of

fluid, and A decreased or negative (developing hypoglycemia of an increased glucose uptake by the limb, but merely states that the rate of glucose uptake by the limb was less than the rate of glucose uptake at sites other than a limb All the foregoing considerations are true only for $(A - V)/A$ values obtained during the period of rapidly falling blood glucose concentrations

Studies in Hepatectomized Dogs

The next step taken was to ascertain whether the liver was causing the narrowing and reversal of the $(A - V)/A$ values by the mechanisms previously discussed, that is, by a decrease in hepatic glucose output and/or an increase in hepatic glucose uptake. A modified form of hepatectomy was devised and developed by one of us (E J. M.) The technical details of this operation will be the subject of a separate article

the 6 hepatectomized dogs that have been studied so far. The accompanying changes in serum potassium and serum inorganic phosphate are shown in FIGURE 8; the fall in serum potassium seems to indicate an increased insulinlike *activity following tolbutamide administration. This did not occur in the* porta caval-shunt dogs. These results demonstrate that, in the absence of the liver, tolbutamide administration results in a hypoglycemia with essentially the same characteristics as that obtained with insulin in porta caval-shunt and normal dogs. These results are therefore consistent with the suggested insulinogenic action of the sulfonylureas and conclusively proved that the sulfonylureas are active hypoglycemic agents even in the absence of the liver.

Serum Amino Nitrogen Studies in Man

Evidence is accumulating that insulin plays a role in the metabolism of amino acids. The plasma level of amino acids is elevated in patients in diabetic acidosis.[29] Luck and Morse[30] have shown that the administration of insulin causes a decrease in plasma amino acids in normal animals. It has been suggested that the decrease in amino nitrogen is mediated through epinephrine for, in the adrenal demedullated rabbits, this fall in amino nitrogen with insulin was not observed.[31] However, Frame and Russell were unable to confirm these observations,[32] and they also noted that the administration of insulin in eviscerated rats depresses the rate of increase of plasma amino nitrogen that consistently occurs following this operative procedure. This observation suggests a peripheral effect of insulin on amino acid metabolism, but it is not known whether insulin promotes the synthesis of tissue protein from

tion of C^{14}-glycine into protein in liver slices.[33] DeMeutter *et al.*[34] have recently found that tolbutamide injected intravenously into human subjects causes a decrease in serum alpha-amino acids, and that this decrease was similar to that produced by insulin in normal human subjects and diabetics. Our observa-

Venous blood specimens were taken at 15-, 30-, or 60-min intervals for 3 to 4 hours. Serum amino nitrogen determinations were performed by the Albanese method.[35]

Results. FIGURE 9 shows that insulin produced a significant decrease in serum *amino nitrogen in both the normal volunteers* and diabetics. In the normals there was a 17 per cent decrease from the initial level and, in the diabetics, a fall of 21 per cent at 3 hours. Insulin and tolbutamide produced a fall in blood sugar that was prompt and significant (FIGURE 10). This fall

tually dropped to less than 50 per cent of the control values. FIGURE 8 shows the arterial and venous blood glucose responses to tolbutamide. Serum potassium and serum inorganic phosphate concentrations are also shown in the same figure. Note that the blood glucose concentrations were again stable prior to the injection of tolbutamide. Tolbutamide injected intravenously

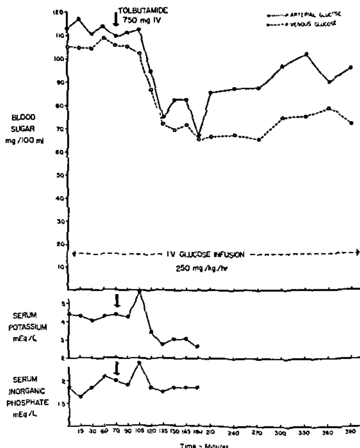


FIGURE 8. Effects of intravenous tolbutamide in a hepatectomized dog. The hepatectomized dog showed an increased $A - V$ difference after tolbutamide similar to that of insulin given to normal or hepatectomized animals.

during the period of stable blood glucose concentrations resulted in a definite hypoglycemia. The developing hypoglycemia was not accompanied by the reversal in arterial and venous blood glucose concentrations that was seen following tolbutamide injection in porta caval-shunt dogs. Determinations of the changes in $(A - V)/A$ during the period of developing hypoglycemia revealed an increased glucose uptake comparable to that obtained with an insulin injection in a port caval-shunt dog. Similar results were obtained in

Discussion

The results presented in the foregoing studies were designed to determine further whether the hepatic action of the sulfonylurea compounds proposed in previous publications could be substantiated [22, 23]. While the results are consistent with such a hypothesis, they do not explain as yet the precise mechanism whereby this hepatic result is effected. However, from our results certain possible mechanisms are suggested.

Interpretation of results of intraportal insulin studies. It has been shown that, unlike insulin, tolbutamide does not increase the disappearance rate of infused D-xylose [22]. Since the premise that tolbutamide affects pancreatic insulin output is, in our judgment, well established, and since this insulin traverses the liver before reaching the peripheral circulation, the question arises as to whether the reason for the lack of effect of tolbutamide on D-xylose might be due to an ineffective amount of insulin reaching the peripheral circulation

[illegible]

in amounts insufficient to produce a marked effect on D-xylose. The possibility that insulin is altered by the liver and is thereby no longer effective in the periphery seems most unlikely. Kaplan and Madison[36] have made observations on the hepatic binding of insulin-I^{131} injected into the portal vein of human subjects undergoing abdominal operation. When the subjects were given no glucose prior to the intraportal injection of insulin, 54 per cent of the injected tagged insulin was bound to the liver in a single transhepatic circulation. With increasing loads of glucose the binding declined progressively from 38 per cent to 7.8 per cent. Thus, hepatic binding of insulin does occur and

[illegible]

D-xylose disappearance, and the studies of Skinner and Madison[37] in rats indicate that during operative stress the hepatic binding of insulin is actually lessened.

[illegible]

by tolbutamide, although there is indirect evidence indicating that this may be the case.

Interpretation of results of porta caval-shunt studies. The use of the arterial-venous glucose difference as a measure of peripheral glucose utilization was suggested by Somogyi[38]. Elrick[39] has advocated the use of the ratio (*arterial*

[illegible]

indwelling catheter, this rate can be presumed to be constant under stable conditions, and the use of the fraction $(A - V)/A$ can be used as a measure

amounted to 71 per cent with insulin and 38 per cent with tolbutamide in the normals The diabetics were less responsive for, with insulin, there was only a 28 per cent decrease even with a larger dose of insulin; with tolbutamide, the maximum fall of 32 per cent occurred at 3 hours.

INSULIN TOLBUTAMIDE

PERCENT OF INITIAL LEVEL

MINUTES AFTER ADMINISTRATION

FIGURE 9 Serum amino nitrogen following insulin and tolbutamide. Both insulin and tolbutamide caused a lowering of serum amino nitrogen in normal and diabetic subjects

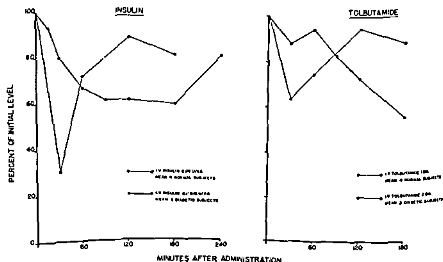


FIGURE 10 Blood glucose following insulin and tolbutamide These graphs show the blood sugar responses to tolbutamide and insulin given to the same subjects as in FIGURE 9

appearance of an increased peripheral glucose uptake, an effect that could not be obtained when the liver was present. The demonstration of an increased peripheral glucose uptake in the hepatectomized animal and the lack of an increased peripheral glucose uptake in the face of hypoglycemia in the porta caval-shunt animal points to the liver as being the preferential site of glucose uptake following tolbutamide administration. Furthermore, the demonstration of an increased peripheral insulinlike action following tolbutamide in the hepatectomized animal supports the view held by many investigators that [illegible]

decreasing or increasing the rate of insulin administration, a high rate resulting in a combined hepatic-peripheral insulin effect with the peripheral insulin effect dominant, and a low rate of insulin administration resulting in a decrease in the peripheral insulin effect so that the hepatic insulin effect becomes dominant.

Interpretation of serum amino-nitrogen studies. It has been shown that insulin does reduce serum amino-nitrogen, and that this effect is both hepatic and peripheral. The results presented in this paper indicate that both insulin and tolbutamide lower the serum amino nitrogen. Bornstein[53] has shown that there is depression of gluconeogenesis with tolbutamide, and this is offered as one explanation for the reduction in blood sugar. Recant and Fischer[33] showed that pretreatment of rats with tolbutamide caused a greater incorpora-

[illegible]

liver may be the primary site of amino acid uptake following tolbutamide administration. Further studies are indicated in order to elucidate whether the liver is the only site of this effect.

Conclusions

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with little or no peripheral participation. In the present studies, however, we were concerned only with the role of the liver in the initiation of the hypogly-

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fonylurea administration. The unexpected lack of an increased peripheral insulin action and the subsequent demonstration of a predominant hepatic insulin action following sulfonylurea administration in these animals led us to conclude that the initial transhepatic circulation of endogenously secreted insulin was not the only factor responsible for the occurrence of the predominant

of glucose uptake Bell and Burns[25] have shown that the intra-arterial injection of insulin results in a wider $A - V$ difference in the limb under study than in the contralateral limb Craig *et al.*[24] have shown that the intra-arterial injection of tolbutamide has no insulinlike action. Employing the arteriovenous glucose difference as an index of insulin activity in acute studies, Purnell *et al.*,[26] using direct arterial and venous blood glucose determination, have shown that tolbutamide does not affect the peripheral utilization of glucose, but Goetz *et al.*,[41] using capillary instead of arterial blood, obtained an increased glucose uptake with tolbutamide. This finding was later supported by the studies of Madison and Unger,[42] who used direct arterial-venous sampling However, these acute studies, for the most part, measured peripheral glucose utilization over the whole experimental period. We have, however, limited the interpretation of our $(A - V)/A$ data in porta caval-shunt dogs to a period of 30 min following tolbutamide, as this limit was found to contain the period of most rapid fall in blood glucose and, we feel, represents the initial hypoglycemic action of the sulfonylureas without the modifying influence of any sig-

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the initial hypoglycemia after tolbutamide administration That the hypoglycemic mechanism involves the liver has been shown in the subsequent experiments in hepatectomized dogs and is supported by the work of other investigators using different techniques[43-47] Madison *et al.*,[48,49] using insulin infusions in porta caval-shunt and normal dogs, have demonstrated that the resulting hypoglycemia is due to a decreased glucose output from the liver that was also associated with an increased glucose uptake by the liver. In our present studies, using higher rates of insulin infusion than those used by Madison and his co-workers, we demonstrated an initial hepatic effect soon followed by a marked peripheral action This difference was presumably due to an overloading of the hepatic insulin-retaining mechanism with resulting increased peripheral effects Our studies also demonstrated the fact that the reduction in glucose output from the liver is not due to the normal physiological route taken by endogenous insulin, but is presumably dependent on a slow release of insulin only.

Interpretation of results on hepatectomized dogs There are now many studies demonstrating that the hypoglycemic action of the sulfonylureas is independent of the presence of the liver[44,46] None of these studies, however, determined whether sulfonylurea-induced hypoglycemia in hepatectomized animals had features different from the sulfonylurea-induced hypoglycemia in intact animals As previously noted in these and other studies, sulfonylurea hypoglycemia has features different from insulin hypoglycemia when insulin is given by injection into a peripheral vein[22,50-52] It is therefore of interest to note that tolbutamide administration in the absence of the liver resulted in the

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. . . Furthermore, upon removing the liver we were able to demonstrate a peripheral initiation of the hypoglycemic responses to sulfonylurea administration.

No conclusions can be drawn from these studies on the degree of peripheral participation in the initiation of sulfonylurea hypoglycemia in the intact or porta caval preparations except to state that, if the periphery does participate in the initial hypoglycemic phase, then this participation must be less in extent than in the liver The maintenance of the hypoglycemic levels after sulfonylurea administration was due to an increased peripheral participation, but the degree to which the liver participated in this later phase could not be determined from these results These results also suggest that the rate of secretion of endogenous insulin controls the relative contributions of the liver or the periphery to the maintenance of hypoglycemia, but that the initiation of the hypoglycemia is due to the predominant but not exclusive participation of the liver Indirect evidence has been presented to indicate that the nature of the hepatic participation is a net increase in glucose uptake by the liver and is, therefore, not due soley to an inhibition of glucose release from the liver cells.

As yet unanswered by these studies are the following possibilities among others Is the hepatic effect due solely to the rate of endogenous insulin secretion after sulfonylurea stimulation? Is there an increased hepatic binding of insulin? Is the marked hepatic effect due to a direct action of the sulfonylureas on the liver cells, making them hyperresponsive to insulin? Has endogenous insulin been altered in some way so that it has an increased hepatic effect? These studies, therefore, reveal the need for further investigations into the factors responsible for the predominant participation of the liver in the initiation of the hypoglycemic response to sulfonylurea administration.

Acknowledgments

Grateful appreciation is expressed to Thomas Casey who participated in the early phases of this work, to Elizabeth Sheldon, for laboratory help, and to Carol Cote for secretarial and art work.

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CLINICAL AND EXPERIMENTAL STUDIES OF INSULIN SECRETION FOLLOWING TOLBUTAMIDE AND METAHEXAMIDE ADMINISTRATION

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Department of Medicine, Johann Wolfgang Goethe University, Frankfurt am Main, Germany

and Related Compounds in Experimental Diabetes in 1957 by The New York Academy of Sciences[1]

As is apparent from the topics of a number of the papers included in the

the failure of increases in peripheral insulin levels in man on this basis[3] However, even in those experiments performed in dogs in which the plasma insulin levels were determined in the portal and the femoral venous blood simultaneously after a single dose of tolbutamide, the increases in plasma insulin in the

gated In view of the fact that a very low dose of this drug is effective in

studies carried out in rats, special consideration was given to the expected differences in insulin levels in portal and peripheral venous blood before and after a single dose of the blood sugar-lowering drugs had been administered and to the determination of pancreatic insulin at the end of the experiments. In both studies the effect of tolbutamide in respect to insulin stimulation was compared with that of metahexamide, each human subject serving as his own control

Studies in Men

and 2 patients had been treated with tolbutamide tablets successfully for 1 and 2 months, respectively, prior to the present investigation. This therapy had been stopped in each case 24 hours before carrying out the sulfonylurea-response test

In the 4 juvenile diabetics, 1 male and 3 females, the metabolic disorder, diagnosed within the first 2 decades of life in each case, had existed at the time of the study for an average period of 13 years. The mean requirement of exogenous insulin amounted to 60 units of a long-acting preparation, the last injection of which was given 24 hours before the administration of the single dose of the antidiabetic drug

In addition to these studies on groups of patients, an individual subject, a 32-year-old female suffering from a pancreatic islet-cell tumor that was proved later at operation, was subjected to the same investigative procedure.

All studies were carried out after an overnight fast. After venous blood samples for determinations of fasting blood sugar and insulin had been obtained at zero time, 25 mg. of tolbutamide/kg. body weight were given in a

kg. body weight

Blood glucose was measured according to the method of Hagedorn and Jensen.[5] The insulin was assayed by using the method of Martin *et al.*[4] This bioassay is based, as mentioned above, upon the oxidation of glucose-1-C^{14} to $C^{14}O_2$ by the rat epididymal adipose tissue It has been slightly modified in the present studies by using diluted serum (1:2) instead of undiluted plasma, since (1) no significant difference in insulinlike activity was found between plasma and serum, and (2) dilution of serum yielded higher and more constant

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epididymal adipose tissue of 3 rats Eight aliquots of 1 serum of a normal subject measured in 1 procedure showed variations in insulinlike activity of ± 40 per cent. The index of precision (lambda) for dose response curves from 62 to 1000 μU./ml. has been 0 29 (FIGURE 1). Normal fasting levels ranged from 135 μU./ml to 680 μU./ml in 15 subjects

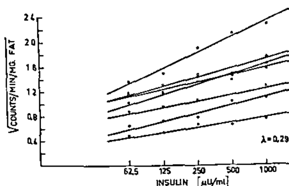


FIGURE 1 The effect of insulin on $C^{14}O_2$ production from glucose 1-C^{14}

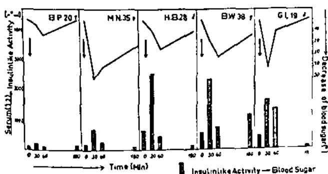


FIGURE 2. The response of normal subjects to a single dose (25 mg./kg., intravenously) of tolbutamide (Orinase).

Results

In 4 of the 5 subjects increases in serum insulinlike activity were observed 30 min after tolbutamide, that is, approximately at or shortly before the maximum decrease in blood sugar concentration One hour after injection

in 3 of the 4 subjects and 180 min. following the tolbutamide in all cases, the insulinlike activity in the serum as well as the blood sugar had returned within range of the initial values.

It thus appears that a correlation exists between the increase in serum insulinlike activity and the fall in blood sugar, expressed as a per cent decrease However, this correlation does not appear to depend upon the absolute rise in insulin activity in the serum, since approximately the same fall in blood sugar has been observed in subjects in whom the insulin levels increased to more than 2000 μU (Case Nos 3 and 4) as in others where only 700 μU /ml serum have been measured as a maximum (Case No. 2).

The changes in serum insulinlike activity and blood sugar concentration induced by the injection of 2.5 mg. metahexamide/kg. of body weight in the same 5 subjects are demonstrated in FIGURE 3. These results were similar to, although less marked than, those obtained with tolbutamide.

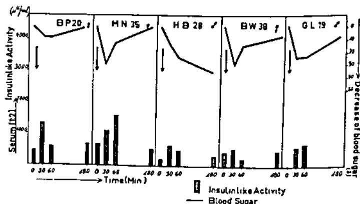


FIGURE 3 The response of normal subjects to a single dose (2 5 mg /kg , intravenously) of metahexamide

This effect is illustrated further in FIGURE 4 which compares only the serum

induced changes in serum insulin

Serum insulinlike activity in 5 elderly diabetics before and after the administration of tolbutamide and metahexamide, respectively FIGURE 5 gives information

normal subjects, again increased in correspondence to the fall in blood sugar within 30 min after injecting 25 mg tolbutamide/kg. body weight. These increases amounted to 2600 and to 4300 μU /ml scrum in the third and fourth patients of this group, respectively, the fasting blood samples of which had yielded the highest insulinlike activity levels in serum at the start of the reference test as well.

In view of the postulated correlation between increases in serum insulinlike activity and decreases in blood sugar it should be noted that the absolute and the relative rises in insulinlike activity again seemed to be of minor importance This may be concluded from the comparative responses of the second and fourth patients in this group In the former, the blood glucose levels decreased

FIGURE 4 Comparative increases in serum insulinlike activity in 5 normal subjects following tolbutamide (Orinase) and metahexamide, respectively

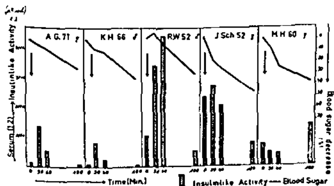


FIGURE 5 The response of elderly diabetics to a single dose of tolbutamide (25 mg /kg, intravenously)

more intensively and rapidly than in all diabetics tested, whereas the insulinlike activity rose only from 470 to 1000 μU./ml. serum; that is, by about 100 per cent. In the latter, a slightly smaller and only slowly progressive decrease in blood glucose resulting in but a primary rise one half hour after the injection of tolbutamide was accompanied by the increase in insulinlike activity from 680 to 4300 μU./ml. (about 600 per cent) within the same interval.

FIGURE 6 shows the results obtained in the 5 diabetics before and after the injection of 2.5 mg. of metahexamide/kg. body weight. They are the same as those observed following the tenfold larger dose of tolbutamide. It may be seen from this figure that 3 hours after the injection of the drug the serum insulinlike activity levels have, in general, returned to the initial ranges, as has been observed in the studies carried out in normal individuals. Contrary to the situation in nondiabetic subjects, however, the *hypoglycemia* still persisted.

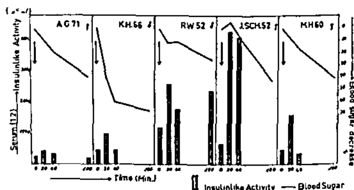


FIGURE 6. The response of elderly diabetics to a single dose of metahexamide (2.5 mg./kg. intravenously).

Something of a consistency in the responses of the individual subjects to the

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diabetics before and after tolbutamide and metahexamide, respectively. The 4 patients previously were shown to be nonresponsive to sulfonylurea therapy. In accord with this finding, no increases in insulin levels and decreases in blood sugar were observed following the administration of a single dose of the sulfonylureas. The insulin levels nearly in the range of the normals and of elderly diabetics are explained by the *comparatively short interval of only* 24 hours between the last injection of exogenous insulin and the institution of the

Figure 7 Comparative increases in serum insulinlike activity in 5 elderly diabetics following tolbutamide and metahexamide, respectively

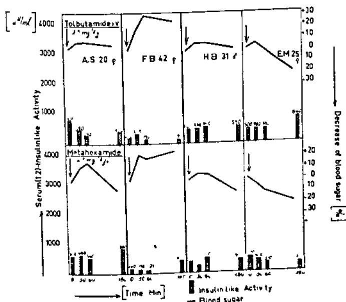


Figure 8 The response of juvenile diabetics to a single dose of tolbutamide (25 mg./kg., intravenously) and metahexamide (2.5 mg./kg., intravenously), respectively.

response test When the insulin administration was discontinued for 48 hours, the serum insulinlike activity was found to be significantly below the values determined in the nondiabetics. However, hyperglycemia and acidosis oc-

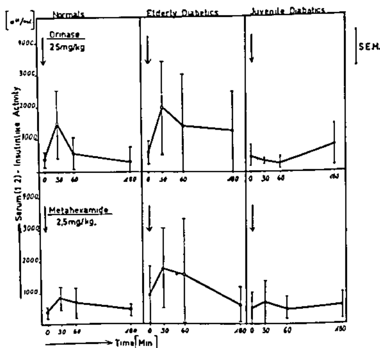


FIGURE 9 Mean of serum insulinlike activity levels in different groups of human subjects before and after tolbutamide (Orinase) and metahexamide, respectively.

In the nondiabetic subjects, on the other hand, the rises in serum insulinlike activity were, in particular following metahexamide, less pronounced. In the elderly diabetics, however, metahexamide has been found to be equivalent in its in

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The juvenile diabetics failed to demonstrate increased insulin activity levels in serum following both tolbutamide and metahexamide.

Serum insulinlike activity associated with an islet-cell adenoma of the pancreas before and after the administration of tolbutamide and metahexamide, respectively. As far as timing of the increases in serum insulin activity and the relation

between the dosages and effects of both drugs are concerned, the same results as in normal subjects and elderly diabetic subjects were obtained in a female patient suffering from an islet-cell tumor of the pancreas. TABLE 1 gives information as to the fasting blood sugar and serum insulinlike activity values in this patient, which were determined on 13 occasions The insulinlike activity levels in serum exceeded the normal range 11 in 13 times Following tolbutamide and metahexamide intravenously (FIGURE 10), the same rapid and marked elevation of serum insulin occurred within 30 min after injection, as has been described above in the normals and elderly diabetics Apparently because of the height of the initial levels, the insulinlike activity increased
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TABLE 1
BLOOD SUGAR AND SERUM INSULIN VALUES IN A FEMALE PATIENT WITH AN ISLET-CELL TUMOR OF THE PANCREAS

Blood sugar (mg %)	Insulinlike activity (μU/ml)	Date
		1958
48	2100	Nov 21
45	1700	Nov 26
85	940	Nov 28
81	210	Nov 29
66	4900	Dec 2
69	1050	Dec 4
70	1500	Dec 5
67	2500	Dec 6
48	2200	Dec 8
75	170	Dec 10
63	3800	Dec 15
		1959
45	2500	Jan 8
62	800	Jan 10

Studies in Animals

Materials and Methods These experiments were designed to study differences between peripheral and portal venous insulin levels before and after the

fed ad libitum until the start of the experiments They were divided into groups of 4 The femoral and portal veins were cannulated by small plastic

after injection. Their pancreas were removed immediately after death for histological and histochemical examinations

Diluted serum (1:5) was used in triplicate for measuring insulinlike activity, as in the studies in humans, according to the method of Martin *et al.*[4] In the cytoplasm of the β-cells the content of SS and SH groups was determined in collaboration with Sandritter and Becker according to the method of Barnett and Seligman.[9, 10] The modification of Bahr[11] was used since it is suitable for evaluating photometrically the staining reactions. As has been shown previously in studies carried out on the pancreas of calves[12] in which the amount of extractable insulin was compared with the cytophotometric absorption figures (Sandritter *et al.*[13]), this method allows us to estimate roughly the intracellular content of insulin and insulin precursors in the β-cells.

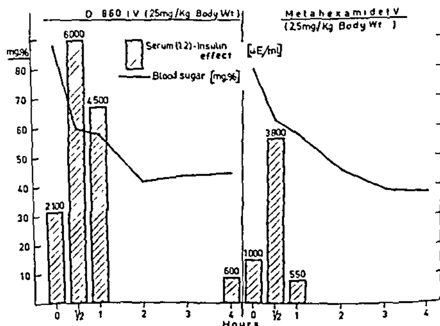


FIGURE 10. Response of a patient with insulinoma to a single dose of tolbutamide and metahexamide, respectively.

Results

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in the former experiments in dogs, the normal serum insulin levels were much higher in portal than in peripheral blood in the rats. Thirty minutes after injection of the sulfonylureas, the decreases in blood sugar and in the content of disulfide groups in the pancreas coincided with remarkable increases in serum insulin in the portal venous blood, whereas only a small rise in insulin levels was observed in general circulation.

Thus, the observation made previously in only two dogs[7] ... the incre...

in blood insulin induced by tolbutamide may be found in the circulation mainly

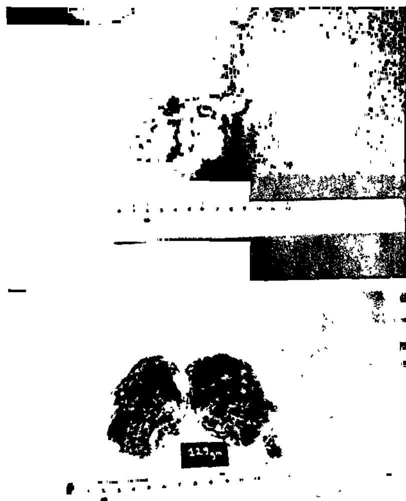


FIGURE 11 Top, an extraordinarily large solid insulinoma (see text) Bottom, tumor opened.

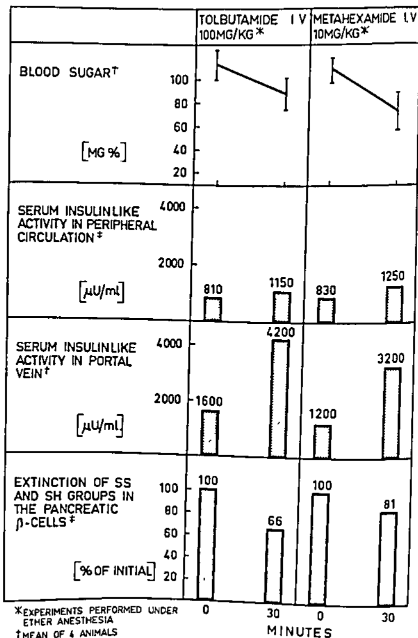


FIGURE 12 Changes in blood glucose, serum insulinlike activity in portal and peripheral circulation, and the content of SS and SH groups of pancreatic β-cells in the rat, determined before and after the administration of tolbutamide and metahexamide, respectively

ureas is based here upon repeated determinations in a total of 12 rats in the tolbutamide and the metahexamide groups, respectively

As regards the rather moderate decreases in blood glucose observed at the 30-min period, it should be understood that ether anesthesia was used throughout the present experiments. In unanesthesized rats the blood sugar underwent an average decrease within the same interval to about 50 per cent of the initial value.

With respect to the comparison between the actions of both drugs on the pancreas, it should be noted that the variations induced by the metahexamide were nearly equivalent to those obtained with the tenfold higher dose of tolbutamide.

Discussion

Our results clearly indicate once again that endogenous insulin is released from the pancreas following the administration of tolbutamide and metahexamide

As far as the studies in man are concerned this conclusion may be reached from the increase in the levels of serum insulin activity established in the peripheral circulation of nondiabetics of tolbutamide-responsive elderly diabetics, and of one patient with a pancreatic islet-cell tumor 30 min after the intravenously administered sulfonylureas. Conversely, no elevations of serum insulin activity were found in the juvenile diabetics not suitable for sulfonyl urea therapy

group.[14a,14b] Their experiments were done blind, in 5 normal subjects and 17 diabetics both before and after tolbutamide by mouth, and the rat hemidia-

tration. A rough calculation shows that the amount of endogenous insulin released into general circulation after sulfonylureas is in the range of exogenous insulin administered peripherally in a dose of about 0.05 to 0.1 U./kg body weight

sive diabetics This prolongation of the hypoglycemia thus should be at-

tributed to an additional mechanism different from that implied in the increased insulin levels in general circulation

Attention should therefore be directed toward the differences between insulin activity levels in the portal and peripheral venous blood (that is, proximal to and distal to the liver) established both before and after administration of tolbutamide This difference was observed only occasionally in our earlier experiments in dogs to such an extent that considerable tolbutamide-induced increases in insulin activity in the portal vein contrasted with the failure of increases in general circulation.[8] It was found in a similar way in the present

less quantitatively pronounced in the different species, permitting only small amounts of insulin to pass into general circulation, as occurred in the rats, for example, whereas considerable concentrations of pancreatic insulin initially reach the circulation after the liver in the human beings If this hypothesis

in the diabetics, be explained by concomitant increases in insulin levels in peripheral circulation Hence, one may be inclined to believe that only the primary phase of the hypoglycemia after tolbutamide is due to the release of endogenous insulin into peripheral circulation, whereas the prolongation of the hypoglycemia is caused by some other, presumably liver-connected, mechanism

Although it would be unwarranted to draw very far-reaching conclusions from these studies performed in only a small number of cases it should be

...g definitely speaks against the opinion, suggested elsewhere,[15,16] that only minor stores of pancreatic insulin are available for immediate release in the pancreas of elderly diabetics who are suitable for sulfonylurea therapy The problem of the pathogenesis of diabetes occurring after the age of 40 (maturity-onset type) becomes even more puzzling, however, when we consider this observation Adding to it the essentially normal or even slightly elevated fasting levels in insulin activity that were found in the elderly diabetics by a number of authors previously[17-20] and again in the studies reported, one may be led to inquire why these patients have been classified as diabetics

...s of the increases in serum insulinlike activity and the decreases in the insulin content of the rat pancreas, metahexamide appears, under the present experimental conditions, to be roughly equivalent to the tenfold larger amount of tolbutamide with respect to stimulation of pancreatic insulin secretion, except in the case of the nondiabetic subjects. No reasonable solution can be offered at this time for the

weaker response that has been noticed in normal human subjects The tenfold greater effect in the elderly diabetics, however, comports with the clinical [illegible]

[illegible] the fact, mentioned before by *Martin et al*,[4] that this bioassay based upon the metabolic activity of the rat adipose tissue does not measure insulin activity, but only insulinlike activity. The second point consists of the problem of insulin inhibitors present in each serum. These factors may also disturb the measurements of insulinlike activity in our studies to a considerable extent

This particular lack of specificity, however, is shared by all bioassays for insulin As to the insulin-inhibiting substances, it should be re-emphasized that the dilution of the sera in our assays resulted in diminishing the activity of the inhibitors. Eventually, they were involved in all determinations of the present investigation, thus interfering uniformly with all measurements Hence, it seems reasonable to assume that the differences established between the insulinlike activity levels measured before and after tolbutamide, on the one hand, and in portal and peripheral circulation, on the other, are real differences. Moreover, the elevated fasting levels in insulin activity measured in the patient suffering from a pancreatic β-cell tumor, and the decreased values in serum insulin found in the juvenile diabetics after a prolonged period of discontinuation of exogenous insulin further support this conclusion

In our opinion, therefore, the fact that the tolbutamide and metahexamide response tests were performed following a single dose of the sulfonylureas deserves more attention In two elderly diabetics only oral treatment was given beforehand. As it may be inferred from studies previously performed in this laboratory on intact calves[12] in which the content of extractable pancreatic insulin was used as an index for estimating insulin release from the [illegible]

correctly.

Summary

[illegible]

fonylureas is due to the release of endogenous insulin into the peripheral circulation, whereas the prolongation of the hypoglycemia is caused by some other mechanism, presumably liver-connected.

This view is supported by the demonstration of differences between insulin activity levels in portal and peripheral venous blood established both before and after one administration of tolbutamide and metahexamide in intact rats. Remarkable sulfonylurea-induced rises in serum insulin in the portal vein contrasted with only small increases in general circulation. With regard to similar observations made previously in other animal species, the concept of some kind of a hepatic binding of endogenous insulin discharged from the pancreas after tolbutamide is supported by these findings and needs further clarification.

On the basis of the increases in serum insulinlike activity established in the elderly diabetics, the patient with an insulinoma, and the rats, as well as the decreases in the insulin (for example, the disulfide group) content of the rat

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DETERMINATION OF METAHEXAMIDE IN HUMAN PLASMA

Arlington A. Forist
The Upjohn Company, Kalamazoo, Mich

Metal
potent,
ureas
sugar concentrations requires a suitable analytic procedure.

Several methods have been reported previously for the determination of antidiabetic arylsulfonylureas Spingler and Kaiser,[1] Forist *et al*,[2] and Bladh and Nordén[3] have described methods for the determination of tolbutamide (1-butyl-3-*p*-tolylsulfonylurea) based on the intense absorption by this compound at 228 mμ The procedure of Toolan and Wagner[4] for chlorpropamide [1-(*p*-chlorobenzenesulfonyl)-3-propylurea] similarly is based on ultraviolet spectroscopy. Visible spectrophotometric methods for tolbutamide determina-

of diazotization and coupling However, in this case the amino group is in the *meta* position relative to the sulfonamide group instead of the *para* position as is true in carbutamide and in the sulfa drugs. Moreover, in metahexamide a methyl group is also present *ortho* to the amino group Because of these structural differences, the behavior of metahexamide in a modification of the Bratton-Marshall procedure[9] has been critically examined and is reported herein

Materials and Methods

The four following *reagents* were used (1) perchloric acid, 10 per cent (a sample of 31 3 ml of 70 per cent perchloric acid is mixed with 300 ml of water), (2) sodium nitrite, 0 1 per cent, (3) ammonium sulfamate, 0 5 per cent, and (4) N-(1-naphthyl)-ethylenediamine dihydrochloride (NED), 0 1 per cent (prepared fresh daily)

Apparatus A Beckman Model B spectrophotometer equipped with 1-cm cells was employed for absorbance measurements

Procedure One ml of plasma (or serum) is mixed with 3 ml. of water, and 4 ml. of 10 per cent perchloric acid is added slowly, with gentle agitation The mixture is swirled occasionally during 15 min and then filtered through Whatman No 40 filter paper (a 2-in funnel with 9 0-cm paper permits recovery of sufficient filtrate for the next step)

On
mi
nit
mate The solution is thoroughly mixed and, after 3 min, 1 ml of 0 1 per cent NED is added The resulting solution is mixed by swirling several times

and then rapidly transferred to the spectrophotometer cell, which should be filled as completely as possible. Absorbance of this solution is determined at 535 mμ versus a reagent blank between 5 and 30 min after the addition of the NED to the sample. A plasma blank (containing no metahexamide) is carried through the same procedure.

The plasma metahexamide concentration (mg/100 ml) is calculated from the equation:

$$M = (A_S - A_B)/a_{535}$$

where

A_S = observed absorbance for the sample at 535 mμ
A_B = observed absorbance for the plasma blank at 535 mμ
a_{535} = absorbance per milligram of metahexamide per 100 ml of plasma for metahexamide added to plasma and carried through the standard procedure (about 0.097).

TABLE 1
DIAZOTIZATION OF METAHEXAMIDE AT 25° C

Time (min)	a_{535}	
	Pseudofiltrate	Plasma filtrate
1	0 070	—
3	0 101	—
5	0 105	—
10	0 105	0 0962
20	0 104	0 0958
30	—	0 0960
40	0 102	—
60	0 100	—

Results and Discussion

application of the method to metahexamide, a careful examination of experimental variables has been made. This has been accomplished by the use of pseudofiltrates (5 per cent perchloric acid) containing metahexamide and, in

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Several methods have been reported previously for the determination of antidiabetic arylsulfonylureas Spingler and Kaiser,[1] Forist *et al*,[2] and Bladh and Nordén[3] have described methods for the determination of tolbutamide (1-butyl-3-*p*-tolylsulfonylurea) based on the intense absorption by this com-

tion have been reported by Spingler ...

... sulfa drugs. Moreover, in metahexamide a methyl group is also present *ortho* to the amino group. Because of these structural differences, the behavior of metahexamide in a modification of the Bratton-Marshall procedure[9] has been critically examined and is reported herein.

Materials and Methods

The

sample

(2) sod

(4) N-(1-naphthyl)-ethylenediamine dihydrochloride (NED), 0 1 per cent (prepared fresh daily)

Apparatus A Beckman Model B spectrophotometer equipped with 1-cm cells was employed for absorbance measurements

Procedure. One ml of plasma (or serum) is mixed with 3 ml. of water, and 4 ml of 10 per cent perchloric acid is added slowly, with gentle agitation The mixture is swirled occasionally during 15 min and then filtered through Whatman No 40 filter paper (a 2-in. funnel with 9 0-cm paper permits recovery of sufficient filtrate for the next step)

Five ml of the proteinfree filtrate is added to a 25-ml Erlenmeyer flask. One ml. of 0 1 per cent sodium nitrite is added and the solution thoroughly mixed Diazotization is allowed to proceed for 10 min, after which the excess

1 ml. of 0 5 per cent ammonium sulfa-

ted and, after 3 min, 1 ml of 0 1 per

ation is mixed by swirling several times

absorbance. This has been traced to an oxidative process For this reason it is recommended that the solution be transferred to the spectrophotometer cell rapidly following addition of the NED to the sample This procedure produces a situation where the surface-to-volume ratio is low and therefore less favorable to air oxidation

Included in TABLE 3 are the results obtained when a sample of metahexamide in a trichloroacetic acid pseudofiltrate, prepared according to Bratton and Marshall,[9] is examined for color production and stability The response is about 90 per cent of that obtained with the perchloric acid system, and the color is considerably less stable. For these reasons, perchloric acid has been employed as protein precipitant

TABLE 4
EFFECT OF AGING OF THE PROTEIN PRECIPITATE ON RECOVERY OF METAHEXAMIDE FROM HUMAN PLASMA

Age of Suspension (min)	a_{546}
15	0 0954
30	0 0966
45	0 0971
60	0 0962

TABLE 5
STABILITY OF METAHEXAMIDE IN A 5 PER CENT PERCHLORIC ACID PSEUDOFILTRATE AT 25° C

Age of solution	a_{546}
5 min	0 104
1 hr	0 105
2	0 105
9	0 105
25	0 105

The effect on the recovery of metahexamide of the exposure of precipitated plasma proteins to perchloric acid has been studied TABLE 4 shows that recovery is constant over a period of an hour Stability of metahexamide in a pseudofiltrate is shown in TABLE 5 No loss is observed over a period of 25 hours, indicating that the proteinfree filtrates need not be analyzed immediately

for at least an additional 25 min. A 10-min. reaction period has been adopted for routine use It can be seen that the response obtained with metahexamide in the plasma filtrate is highly reproducible and is about 91 per cent of the response obtained with standards in the pseudofiltrate

To avoid reaction with the coupling reagent (NED), excess nitrite is destroyed by the addition of ammonium sulfamate. To define allowable variations in time at this step, stability of the diazonium salt in the reaction mixture

TABLE 2
STABILITY OF DIAZOTIZED METAHEXAMIDE IN THE PRESENCE OF EXCESS AMMONIUM SULFAMATE AT 25° C.

Age of solution (min)	a_{s4s}*
1	0 105
3	0 105
5	0 104
10	0 105
20	0.104
40	0 103
60	0 104

* Five per cent $HClO_4$ pseudofiltrate

TABLE 3
STABILITY OF THE AZO COMPOUND FROM METAHEXAMIDE

Time after NED addition (min)	a_{s4s}*		
	Pseudofiltrate	Plasma filtrate	TCA pseudofiltrate
3	0 102	0 0939	0 0940
5	0 104	0 0958	0 0937
7	0 104	0 0960	—
10	0 104	0 0962	0 0925
15	0 104	0 0962	0 0913
20	0 104	0 0964	—
25	0 103	0 0964	—
30	0 103	0 0964	—
45	0 103	0 0966	0 0893
60	0 101	0 0964	0 0893

* Sample held in 1-cm spectrophotometer cell

containing excess ammonium sulfamate has been examined TABLE 2 shows that destruction of nitrite is complete within 1 min, and that the diazonium salt is stable for at least 1 hour A 3- to 5-min reaction period has been employed for routine use, but considerable flexibility is possible.

Rate of formation and stability of the azo compound following addition of the NED has been determined. TABLE 3 shows that 5 min. are required for maximum color formation in both the pseudofiltrate and the plasma filtrate Some loss occurs in the pseudofiltrate after about 30 min in the spectrophotometer cell. Color stability is somewhat greater for the plasma filtrate, with no loss occurring over a period of an hour However, solutions left in the reaction vessel (a 25-ml Erlenmeyer flask) show considerable run down in

Summary

A modification of the Bratton-Marshall[9] procedure for the determination of aromatic amines has been developed for the determination of metahexamide in human plasma. Greater color stability and somewhat higher sensitivity have resulted from the substitution of perchloric acid for trichloroacetic acid as the protein precipitant. Experimental variables have been thoroughly evaluated. Under the conditions described, recovery of added metahexamide from human plasma over the range 1 to 5 mg./100 ml. has been 100.4 ± 1.9 per cent (mean ± standard deviation).

dicated an apparent metahexamide concentration of 0.1 to 0.2 mg./100 ml. Therefore, for maximum accuracy, especially at low levels, a plasma blank should be run and the appropriate correction applied as described above.

Application of the method to the determination of metahexamide added to human plasma has given the results shown in TABLE 6. The mean recovery

TABLE 6
RECOVERY OF METAHEXAMIDE ADDED TO HUMAN PLASMA

Added (mg./100 ml.)	Found (mg./100 ml.)	Recovery (%)
	Plasma 1	
0.94	0.95 0.96	101.1 102.1
1.89	1.95 1.96	103.2 103.7
2.83	2.73 2.82	96.5 99.6
3.78	3.71 3.78	98.1 100.0
4.73	4.64	98.1
	Plasma 2	
0.94	0.93	98.9
1.89	1.94 1.89	102.6 100.0
2.83	2.87 2.84	101.4 100.4
3.78	3.82 3.81	101.1 100.8
4.73	4.69 4.71	99.2 99.6

Mean ± standard deviation 100.4 ± 1.9%

plus or minus standard deviation of 100.4 ± 1.9 per cent indicates excellent accuracy and precision.

The procedure as presented is useful over the range 0.2 to 8 mg. metahexamide/100 ml. plasma. Greater sensitivity, if needed, should be possible by employing a larger plasma sam
proteinfree filtrate, provided, th.
is rapid, accurate, and precise.
only other aromatic amino compounds interfere. Direct extension of the method to the analysis of urine is possible, recognizing that metabolites as well as intact metahexamide are determined.

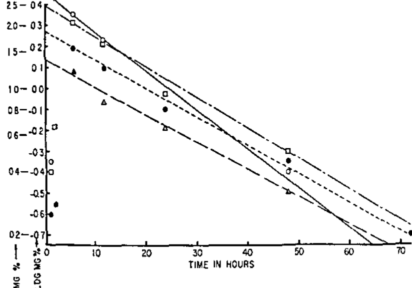


FIGURE 1 Typical plots of log metahexamide in milligrams per cent versus time in hours for individual patients on 200 mg, orally Symbols O, patient S'h, △, patient Br,, □, patient Ja, and ●, patient Pe

TABLE 1

Milligrams
le Dose

Name	0	½	1	2	6	12	24	48	72	96
Du	0 1[a]	1 3	1 4	1 7	1 35	1 3	1 05	0 43	0 23	0 12
	108[b]	88	80	86	113	108	90	106	97	100
Wa	0 05[a]	0 05	0 1	0 2	1 25	85	0 25	0 15	0 12	0 10
	92[b]	88	96	80	92	100	94	108	95	93
S'h.	0 1[a]	0 25	0 55	2 82	2 35	1 80	1 05	0 50	0 22	
	113[b]	103	100	94	98	103	100	113	98	
Du	0 1[a]	0 45	0 98	80	0 65	1 05	0 62	0 35	0 1	0 05
	110[b]	170	109	103	98	92	100	94	113	103
Mu	0 2[a]	0 4	0 9	2 0	1 75	1 1	0 80	0 15		0 1
	108[b]	110	105	119	103	119	103	125		122
Ja	0 15[a]	0 3	0 55	0 80	2 2	1 8	1 1	0 65		0 21
	114[b]	92	95	110	106	116	103	110		110
Br	0 05[a]	0 1	0 1	0 15	1 25	0 91	0 70	0 37		0 08
	124[b]	119	108	98	106	103	100	116		
Do	0 05[a]	0 08	0 1	0 55	1 10	0 70	0 37	0 25		0 05
	122[b]	129	110	106	106	98	113	103		
Si	0 08[a]	0 09	0 10	0 15	1 12		0 65	0 15	0 08	0 05
	93[b]	98	90	103	96		103			
Op	0 07[a]	0 10	0 15	0 22	1 30		0 88	0 10	0 07	0 03
	103[b]	86	92	98	98		96			
Pe	0 10[a]	0 25	0 35	0 38	1 65	1 35	0 90	0 55	0 30	0 15
	125[b]	153	149	100	92	88	95	93	103	
Ha	0 15[a]	0 30	1 22	1 70	1 15	1 00	0 72	0 20	0 15	0 10
	88[b]	120	94	82	103	91	85	93	98	

DETERMINATION OF THE HALF LIFE OF METAHEXAMIDE IN NORMAL HUMANS

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Metahexamide [1-(3-amino-*p*-tolylsulfonyl)-3-cyclohexylurea] is a new experimental antidiabetic sulfonylurea Before extensive clinical evaluation of this new compound on diabetics, the time for halving blood levels of metahexamide (that is, the effective half life) was determined. This paper summarizes the clinical investigation (L L M.) and the data analysis (E.R G).

Procedure

Twelve normal nonfasting subjects were used; 9 were males and 3 were females All cases but patient Wa. were white, all but one were ambulatory volunteers who carried on their normal daily activities except for the times at which specimens were taken The exception was patient Wa , hospitalized with bilateral tibial fractures and, at that time, at bed rest. The subjects were all seen

200 mg

this, blo

96 hours

by the method described by Forist, elsewhere in this monogram. This method is a modification of the Bratton-Marshall procedure,[1] the nonfasting blood sugar level was determined by the method of Folin and Wu

Results and Discussion

Typical plots of the log metahexamide blood concentration versus time in hours are shown in FIGURE 1 The rates of elimination from the blood are summarized in TABLE 2 as based on the data in TABLE 1 corrected for the zero-hour value Included in TABLE 2 are the slopes of the semilogarithmic plots, the derived rate constants, k; the half lives, $t_{1/2}$, of metahexamide elimination from the blood, the number of points serving as a basis for a rate estimate, the hour of maximum value attainment from the 1-, 2-, and 6-hour values, and the maximum value in milligrams per cent.

The basic equation for first-order elimination from the blood is.

$$\log A = -(k/2.303)t + \log A_0 \quad (1)$$

where A_0 is the amount of drug at zero time (extrapolated) and the first-order rate constant, k, is in units of reciprocal time, that is, hour^{-1}. The slope, $S = k/2.303$, can be transformed to the half life $t_{1/2}$, the time at which half the drug is eliminated, by the expression

$$\frac{-2.303 \log 0.5}{k} = \frac{6.91}{k} = \frac{2.30}{S} = t_{1/2} \quad (2)$$

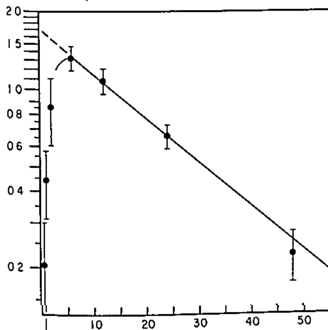
by subtraction of the "zero" value, as the "mean" value for all patients at the time of oral administration The vertical lines in FIGURE 2 represent the standard deviation of the mean.

The large standard deviations at the 0 5-, 1-, and 2-hour values are due to

TABLE 3
STATISTICS OF METAHEXAMIDE BLOOD LEVELS*
(Based on the Mean Value of All 12 Nonfasting Patients at a Given Time After Oral Administration of 200 mg)

Time in hours	Mean mg per cent $\bar{X}$	Standard deviation σ	Standard deviation of mean† $\sigma\sqrt{n}$
0 5	0 206	0 329	0 095
1	0 442	0 447	0 129
2	0 856	0 859	0 248
6	1 33	0 47	0 14
12	1 08	0 36	0 12†
24	0 658	0 245	0 071
48	0 225	0 175	0 051

were not taken and $n = 10$, $D F = 9$



It is our opinion that, although a rate constant or half life derived from the pooling of individual's data obtained at the same time after administration may serve as a value useful in comparison with another drug, yet it has little significance in estimating human variability in maintenance of drug blood levels. A better choice of data treatment is to determine half-life values of the individuals.

Such data are given in TABLE 2. The mean half life is 18.2 hours, the standard deviation σ of an individual being 5.7 hours. The estimated tolerance

TABLE 2
SUMMARY OF RATES OF METAHEXAMIDE BLOOD LEVEL LOSS IN PATIENTS*

Patient	Slope of log blood level versus time	Estimated rate constant for elimination from blood k (hr^{-1})	Half life in blood ($t_{1/2}$ in hours)	No. of points serving as basis of rate estimate**	Hr of max value from 1-, 2-, 6-hr points	Max value, mg per cent
Du	0 0158	0 0564	19 0	6†	2	1 60
Wa	0 0478	0 110	6 3	3††	6	1 20
S'h	0 0188	0 0433	15 9	6‡	2	2 72
Du	0 0153	0 0352	19 6	3	?‡‡	0 95
Mu	0 0220	0 0507	13 6	4§	2	1 80
Ja	0 0146	0 0337	20 6	4‡	6	2 05
Br	0 0132	0 0304	22 6	4‡	6	1 20
Do	0 0241	0 0556	12 4	3§	6	1 05
Si	0 0145	0 0534	20 7	2§§	6	1 04
Op	0 0106	0 0245	28 3	2§§	6	1.23
Pe	0 0137	0 0316	21 9	5‡	6	1 55
Ha	0 0175	0 0404	17 1	4§	2	1 55

[illegible] indicative of experimental [illegible]tion

§ Fair first-order fit

§§ Quality of fit unknown, 2 points can only estimate 1 straight line

limits[2] within which 95 per cent of the population of half lives will fall is 18 2 ± 13.1

TABLE 2 sh[illegible] hours, but in [illegible] mg per cent f[illegible] cent

An extreme value in TABLE 2 is that of patient Wa., the only possible exception to the criterion of "healthy" adults, a Negro hospitalized with bilateral tibial fracture, but no other pathology

The other method for treatment of the data is shown in TABLE 3 and plotted in FIGURE 2. This treats the blood level data, after correction for background

average half life of 18 2 hours, with the standard deviation among individuals of plus or minus (±) 5 7 hours and with a standard deviation of the mean of 1 6 hours It can be concluded that metahexamide has, within biological variations, 4 to 5 times the half life of tolbutamide in the blood and approximately one half the half life of chlorpropamide

Acknowledgment

Gratitude is expressed to Homer Kesten and George Mackewen, of the laboratory of the White Plains Hospital for the blood level determinations

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the different absorption rates among the individuals (see TABLE 1). Absorption has been virtually completed by 6 hours; actually, only 3 points (6, 12, and 24 hours) are valid for the estimation of the half life of metahexamide in the plasma The 48-hour value is significantly greater than zero, but it must be realized that an absorbance of 0 020 indicates an 0 2 mg. per cent of metahexamide, since Forist's method was designed for the range 0 to 5 mg. per cent of metahexamide in the blood

Inspection of the clinical data at the "zero" hour clearly shows an "apparent" metahexamide concentration of 0 1 to 0.2 mg. per cent in plasma containing no metahexamide This blank value is consistent with Forist's estimate.

An important item to remember is that any aromatic amine will provide a positive assay artifact, since it will react in the Bratton-Marshall test.[1] For example, the oral administration of phenacetin (acetophenetidine), C_2H_5O-C_6H_4-$NHCOCH_3$, a component of phenacetin, aspirin, and caffeine (PAC),

some factor other than gastric absorption is rate-determining in the appearance of metahexamide in the blood At a gastric *p*H of 2 or 3, the solubility of metahexamide is about 20 to 10 mg./100 cc. At a 200-mg. dose, the total water content of the stomach would have to be 1 to 2 l for the material to be totally dissolved, on the assumption of instantaneous dissolution.

It thus follows that complete dissolution will probably not occur until the *p*H 6 or 7 of the intestine is reached, where the solubility is 60 to 500 mg./100

*p*H of minimum

mg./100 cc.

, but the greater

of metahexamide In general, charged compounds are absorbed with difficulty by the gastrointestinal mucosa The point of minimum solubility (probably in the duodenum) is the *p*H of maximum concentration of noncharged molecules and the point of maximum ab

tio

that is, a relatively prolonged absorption time attributable to the unique inter-

ac

tr

that of metahexamide at 200 mg oral dose is 16 to 20 hours. Thus it may be concluded that metahexamide has 4 to 5 times the duration of tolbutamide in the blood on oral administration The half time of chlorpropamide is approximately 32 hours;[4] thus the half life of metahexamide is about one half that of chlorpropamide.

Summary

Pseudo first-order rate plots of metahexamide elimination from human blood on 12 nondiabetic, nonfasting, adult humans given 200 mg orally showed an

virtually free of toxicity as is tolbutamide Several years ago I investigated the halogen derivatives of sulfonylureas p-chloropropyl- and p-chlorobutyl-sulfonylurea, respectively, are more potent than tolbutamide but at the same

ring

TABLE 1
TOLBUTAMIDE IN RATS, 9 MONTHS, $n = 5$

Dose	(mg/kg, orally)			
	250	500	1000	2000
Liver	no pathological findings	no pathological findings	no pathological findings	slight fatty liver
Kidney	no pathological findings	no pathological findings	no pathological findings	no pathological findings
Heart	no pathological findings	no pathological findings	no pathological findings	no pathological findings
Other organs	no pathological findings	no pathological findings	no pathological findings	hyperplasia of the thyroid

TABLE 2
TOLBUTAMIDE IN RATS, 9 MONTHS, $n = 5$

Dose	(mg/kg, orally)				Control
	250	500	1000	2000	
Hemoglobin	normal	normal	normal	normal	—
Erythrocytes	normal	normal	normal	normal	—
Leukocytes	normal	normal	normal	normal	—
Diff count	normal	normal	normal	normal	—
Urine					
Sugar	←without pathological findings→				—
Protein	←without pathological findings→				—
Sediment	←without pathological findings→				—
Bile	←without pathological findings→				—
Liver glycogen (percentages)	4 3	2 9	2 7	2 5	3 3
Glucose tolerance	—	—	—	nondiabetic	—

TABLE 3
TOLBUTAMIDE IN DOGS, 9 MONTHS, $n = 5$

Dose	(mg/kg, orally) 100
Liver	[illegible]

Part IV. The Status of Oral Agents in the Therapy of Diabetes

PHARMACOLOGICAL STUDIES OF THE SULFONYLUREAS

Alfred Bander
Pharmacological Department, Farbwerke-Hoechst A. G, Frankfurt (M) Hoechst, Germany

A good basis for further development of oral antidiabetics is found in toxicological experiences gained with the preparations tolbutamide and carbutamide, already on the market I am reporting results of long-term experiments with high dosages of tolbutamide These results were obtained in cooperation with Scholz *et al* [1-3] Four years of clinical experiences have proved the accuracy of our previous toxicological prognosis based on results of studies on animals, namely, the absence of toxicity in these preparations. Rules regarding duration, intensity, and variation of toxicological tests with animals have been postulated recently also by Lehman.[4] These rules must be recognized, as they are based on experience These criteria should be applied when new oral antidiabetics are to be developed

After the introduction of insulin, death in the majority of diabetics is not the direct consequence of the disturbed metabolism, but is the result of the late complications, particularly of the vascular system. In addition to possessing therapeutic effectiveness, modern antidiabetics should not augment complications or provoke new ones A prognosis regarding possible side effects can be made only when the drug in question has been administered over a prolonged

logical changes This limit dose should be far higher than the therapeutic dose because very strict measures must be applied in drugs intended for administration over a period of decades as compared to those designed for short-term treatment

The therapeutic dose of tolbutamide for human beings is approximately 25 mg./kg when given orally Therefore I have given rats 250, 500, 1000, and even 2000 mg /kg orally over a period of 9 months. Mix-bred animals were used in air-conditioned rooms and received a standard diet. The drug was fed only by stomach tube Dogs received 100 mg /kg orally over a period of 9 months Higher dosages cannot be given to dogs for this length of time, since the animals would die of acute hypoglycemia. The results are shown in TABLES 1, 2, and 3 We have done considerably more studies in general pharmacology, but the results are of minor importance in the present context.

For us these studies are the standard against which all other hypoglycemic drugs are to be tested In Hoechst, Germany, more than 1000 hypoglycemic substances have been synthesized thus far. A few of these substances are more potent than tolbutamide, but up to now not one was found to be as

this case, also, increased potency is connected with decreased chronic tolerability despite the fact that the acute toxicity of metahexamide is within the range of tolbutamide. The results obtained from my standard tests are shown in TABLES 7, 8, and 9

TABLE 7
METAHEXAMIDE IN RATS, $n = 10$

Dose	(mg/kg, orally)				
	100	250	500	1000	2000
General tolerance	after 2½ months, 1/10 died, after 3 months, 5/10 sacrificed	after 2 weeks, 2/10 sacrificed	within 1 to 2 weeks, 10/10 died, resp killed before spontaneous death	within 7 to 10 days, 5/5 died, resp killed before spontaneous death	after 2 to 5 days, 5/5 died, resp killed before spontaneous death
Liver	fatty degeneration ++, necrosis +	fatty degeneration +++, necrosis +	fatty degeneration ++++, necrosis ++	fatty degeneration ++++, necrosis ++	fatty degeneration +++ necrosis
Kidney	fatty + nephrosis	fatty + nephrosis	fatty ++ nephrosis	nephrosis	nephrosis
Heart	without pathological findings	1/10 diff fatty infiltration +	4/5 diff fatty infiltration +	2/5 diff fatty infiltration ++	without pathological findings

TABLE 8
METAHEXAMIDE IN RATS

Dose	(mg/kg) 100	Control
Hemoglobin	normal	—
Erythrocytes	normal	—
Leukocytes	9100	12200
Diff count	normal	—
Urine		
Sugar	negative	negative
Protein	+	±
Sediment	negative	negative
Liver glycogen (percentages)	1 05	6 7
Glucose tolerance	diabetic	nondiabetic

Until now I have observed that metahexamide is tolerated over a sufficiently prolonged period, but not without damage, in doses as low as 100 mg/kg in

Another product is metahexamide. This compound was developed in a joint research program together with C. F. Boehringer, Mannheim, Germany. In human beings the potency of this drug is as much as 10 times that of tol-butamide, the therapeutic maintenance dose is approximately 2.5 mg/kg. In

TABLE 4
CHLORPROPAMIDE IN RATS, $n = 5$

Dose	(mg/kg, orally)		
	250	500	2000
General tolerance	duration 4 weeks, 0/5 died	duration 4 weeks, 3/5 died	after 2 days 5/5 died
Liver	fatty + degenerative proc	fatty ++ degenerative proc.	no histology because of decay
Kidney	1/5 nephrosis	fatty tubuli	
Heart	without pathological findings		

TABLE 5
CHLORPROPAMIDE IN RATS, $n = 5$

Dose	(mg/kg, orally)		
	50	100	Control
Hemoglobin	normal	normal	—
Erythrocytes	normal	normal	—
Leukocytes	normal	normal	—
Diff count	normal	normal	—
Urine			
Sugar	without pathological findings		—
Protein	without pathological findings		—
Sediment	without pathological findings		—
Bile	without pathological findings		—
Liver glycogen (percentages)	4 2	3 4	4 8
Glucose tolerance	diabetic	diabetic	nondiabetic

TABLE 6
CHLORPROPAMIDE IN DOGS, $n = 3$

Dose	(mg/kg, orally)	
	10	50
General tolerance	2/3 died	2/3 died
Liver	fatty +	fatty ++
Kidney	nephrosis	nephrosis
Heart	fatty +	fatty +++
Blood	normal	normal
Urine	without pathological findings	
Glucose tolerance	diabetic	daibetic

ROLE OF ORAL BLOOD SUGAR-LOWERING AGENTS IN THE MANAGEMENT OF DIABETES

Robert F. Bradley

The Joslin Clinic and New England Deaconess Hospital, Boston, Mass

Four short years span the introduction and extensive clinical trial of sul- [illegible]

[illegible] the attainment of blood sugar levels that are within the normal range (normo- or euglycemic). Since the sulfonylureas and biguanides are not insulin and do not *directly* produce metabolic effects comparable to those that follow insulin administration, the clinician may simply look upon their actions as those by which a certain minimum of insulin, available endogenously or exogenously, appears to be more effective.

The steady stream of pills available to thousands of diabetics has been composed of 4 sulfonylurea and 3 biguanide compounds. With the possibility [illegible] current [illegible] of significant toxicity, will offer some po[illegible] or simpler management without jeopardizing the many victories won for the [illegible] eserving [illegible], that if [illegible] it must be at a level of incidence and severity much lower than if there were no other means of treatment.

Sulfonylurea Compounds

The sulfonylurea compounds have undergone sufficient clinical trial to [illegible] butamide (Orinase†) has gained [illegible] 1). Currently the newer agents, [illegible] 2), because of enhanced potency appear to have broadened the field of sulfonylurea usefulness in responsive diabetic patients [illegible]

[illegible] but is most rapid for metahexamide.[5] Delayed excretion and durations of [illegible]

§ Chas. Pfizer & Co., Inc., Brooklyn, N.Y.

TABLE 9
METAHEXAMIDE IN DOGS

Dose	(mg /kg , orally)		
	10	25	50
Duration	2 months	3 weeks	1 week
General tolerance	0/5 died, 1 animal sacrificed, anorexia	2/5 died, anorexia	after 7 days, 5/5 in poor general condition, 1 animal sacrificed
Liver	fatty + (retention of bile as in ascending cholangitis) cirrhosis	fatty ++ cirrhosis	fatty +++
Kidney	nephrosis	nephrosis	nephrosis
Heart	without pathological findings	diff fatty + infiltration	dif. fatty + infiltration
Blood	Ery, Leuk, Hb, Diff count normal	normal	—
Urine	— bile ±	protein + bile +	protein ++ bile +
Glucose tolerance	diabetic	diabetic	—

dosages in rats and dogs are under way, but are not yet finished Therefore, a final evaluation from the toxicological point of view cannot be made at the moment

Findings observed in animals thus far, however, indicate that clinical trials should be performed with particular caution and criticism, and over a sufficiently extended period of time All possible advantages of a drug should be regarded as secondary to the patient's safety. Increased toxicity should never be the price paid for a reduced dose

References

1 SCHOLZ, J & A BANDER 1956 Deut med Wochschr 81: 825–826
2 BANDER, A & J SCHOLZ 1956 Deut med Wochschr 81·889–891
3 BANDER, A, A HAUSSLER & J SCHOLZ 1957 Deut med Wochschr 82· 1557–1564
4 LEHMAN A J 1959 Drug Trade News No 4

continue to be those with maturity-onset, stable, so-called "mild" diabetes. In general, "severity" of diabetes is greater the earlier the age of onset,[16] so that selection of diabetics according to onset later in life (age 40 and older), as well as upon insulin requirement (40 U. and preferably 20 U. per day or less) becomes a guide to "mildness" that has far greater accuracy than attempting to substitute a sulfonylurea for a given number of units of insulin. By doing so, many physicians would avoid the trap of treating the early juvenile case in partial clinical remission only to see the apparently beneficial effects swept aside in the ensuing weeks and months as the juvenile pattern continues

under age

after many

months of inadequate tolbutamide response. Several were in ketoacidosis, 1 with coma. Two young men, ages 21 and 23 at onset of diabetes, have come for treatment in the past 2 months after ineffective diet and tolbutamide therapy. Each had lipemia retinalis, and 1 came for diagnosis of typical

TABLE 1

ADMISSION FINDINGS IN TWO DIABETICS NOT RESPONDING TO TOLBUTAMIDE

Patient	Sex	Age at onset of diabetes (years)	Duration of treatment with tolbutamide (years)	Blood sugar (mg per cent)	CO_2 (ml per L)	Blood cholesterol (mg per cent)	Blood cholesterol after insulin (mg per cent)
V C	M	23	1 5	400	16	1275	768*
W M	M	20	1	375	25	2066	206†

* One week

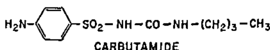
† Six weeks

xanthomatosis. Until shortly before admission both stated they felt well and denied diabetic symptoms of sufficient severity to cause undue concern. Serum from each young man was creamy. Laboratory findings confirmed uncontrolled diabetes, mild to moderate ketoacidosis, and severe disturbances in

or retinopathy are present in an active phase. Where these complications

action for chlorpropamide (half life, 35 to 40 hours[6]) and metahexamide (half life, ±30 hours[6,7]) that are longer than those noted for tolbutamide (half life, ±6 hours) further increase the potency of the former when used in maintenance studies

Mechanism of Action Little or no evidence suggests that the sulfonylureas lower blood sugar by a direct, insulinlike action. However, ample data support the concept that they act on the pancreas to stimulate the production or release of insulin[8,9] and on the liver to inhibit glucose formation or release.[10,11] The continuing controversy concerning the relative contributions of these two effects of the sulfonylureas indicates that much has yet to be learned concerning their mechanism of action However, in terms of their clinical application the demonstration of pancreatic islet stimulation[12,13] and of promotion of



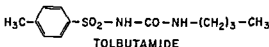
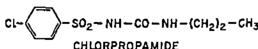


FIGURE 1



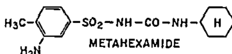


FIGURE 2

endogenous insulin secretion by sulfonylurea compounds has given considerable impetus to their extensive trial in many thousands of diabetics.

Although some effects of tolbutamide on protein and lipid metabolism are similar to those produced by insulin, such as decrease in blood nonesterified fatty acids[14] and serum amino acids[15] in humans who show a fall in blood sugar after drug administration, more study is needed to determine the extent to which response of total diabetic metabolism to sulfonylureas is comparable to that obtainable with insulin.

Sulfonylurea Compounds in the Management of Diabetes

Successful management of diabetes with any sulfonylurea compound will depend in large measure upon proper selection of patients for trial, and on education of the selected individuals concerning the importance of diet and long-range aims in total treatment.

Selection of patients. Patients responding adequately to a sulfonylurea

TABLE 2

CRITERIA OF CONTROL*

Relation to food	Degree of control†		
	Good Blood sugar‡ mg /100 cc	Fair Blood sugar‡ mg /100 cc	Poor
Fasting	110	130	All others
1 hour, p c	150	180	
2 hours, p c	130	150	
3 hours, p c	110	130	
Urine sugar in 24 hours	2 gm or less	5 gm or less	

values

TABLE 3

RESULTS OF LONG-TERM USE OF TOLBUTAMIDE

	No of patients	Percentages of total
Good control of hyperglycemia and glycosuria	491	55 0
Fair control of hyperglycemia and glycosuria	121	13 6
Primary failures (within first month of treatment)	199	22 3
Secondary failures	63	7 1
Other	18	2 0
Total	892	100 0

Long-Term Use of Sulfonylurea Compounds

Tolbutamide. Since tolbutamide (Orinase) is the only sulfonylurea having extensive clinical trial for more than one year in the United States, current

sulfonylureas will

), and now includes

a total of 1500 diabetic patients, of whom an estimated 1000 have been on maintenance studies for longer than 1 month to more than 3 years Although

have been present for some time and are stable, their presence in the responsive patient has not been a contraindication. In that age group of diabetics who respond best to sulfonylureas, atherosclerotic occlusive disease of coronary, peripheral, and cerebral arteries is far more common. For individuals with such conditions these agents have seemed desirable because the blood sugar often can be kept normal or nearly so without the concern felt by many that insulin-induced hypoglycemia would produce myocardial infarction or ag-

responded well No specific duration can be used reliably to demarcate the successes and failures.

Certain patients selected by the above criteria are not controlled by sulfonylureas The safest means of eliminating all "immediate failures" (those diabetics rapidly developing uncontrolled diabetes and ketoacidosis when insulin is omitted for more than 24 hours[18]) and most "primary failures" (those occurring in the first month of treatment) is to measure the blood sugar response at ±4 hours following a single, adequate loading dose of sulfonylurea.[19]

The "sulfonylurea response" test is by no means infallible. About one fourth of the diabetics having inadequate drop in blood sugar will be found, many days or weeks later, to be doing well on maintenance therapy. On the other hand, later "fair" results or "secondary" failures (after one month of sulfonylurea treatment) occur more often in patients with borderline responses to the test

Chlorpropamide does not yield consistent reductions in blood sugar level within the number of hours considered reasonable for testing Therefore, trial with this agent is based on day-to-day maintenance treatment. Metahexamide thus far has yielded surprisingly consistent blood sugar reductions in the responsive patient 4 to 6 hours after administration of the loading dose

Evaluation of treatment Standards used in evaluating degree of control obtained with any sulfonylurea will influence significantly the degree of success reported. The seemingly rigid criteria originally adopted for study of Joslin Clinic patients taking carbutamide[20] are equally worthwhile in evaluating tolbutamide (TABLE 2) Although arbitrarily chosen, these strict standards are not meant to create an insurmountable path to success for the sulfonylureas There are three important reasons for their use

(1) Early in the course of sulfonylurea trials it was found that the *responsive* diabetic would have blood sugar and urine values consistently in or near the normal range, this has continued to be true

(2) Patients not qualifying by these criteria often profess subjective well-being, yet increasing tolbutamide dosage, or omitting it entirely, frequently produce no change in blood sugar levels; this suggests that tolbutamide has no significant effect in these individuals

(3) In the absence of other means of demonstrating antidiabetic action—that is, prevention of late complications—standards of metabolic control should be strict.

Serious toxicity possibly related to tolbutamide has occurred in 4 patients In 1 woman with pre-existing cirrhosis and esophageal varices, jaundice lasting 2 weeks appeared 3 months after tolbutamide was begun A second

hepatitis, suggesting infectious origin These changes were interpreted* as being not comparable to those seen in a case of liver damage apparently due to carbutamide

With far better documentation of its relation to tolbutamide, an allergic type of purpura occurred in a 63-year-old man and a 56-year old woman After taking the drug for 1 and 6 months, respectively, they developed lassitude, petechial eruptions of the extremities, fever, and hepatosplenomegaly A reduction in platelets, elevated Bromsulphalein retention and alkaline phos-

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The

results, to be reported by Mirsky and Krall,[23] indicated that antiplatelet antibody formed in the presence of tolbutamide produced the clinical picture Administration of hydrocortisone and omission of tolbutamide produced rapid recovery in the male patient and subsequently it became possible gradually to omit hydrocortisone without recurrence The woman's symptoms and signs disappeared gradually after omission of tolbutamide.

sufficiently long to warrant considerably increased confidence in the drug's

Serious or prolonged

*By William Meissner, Department of Pathology, New England Deaconess Hospital, Boston, Mass

†By James Tullis and Hugh Pyle, Protein Foundation, Boston, Mass

‡Department of Clinical Investigation, The Upjohn Co, Kalamazoo, Mich.

patients studied by Hadley *et al*,[22] the same or a slightly higher percentage of the maturity onset type of diabetic patients achieved adequate diabetic control Their preliminary report demonstrated good control in 68 per cent and fair control in 15 per cent of the patients so selected.

The current status of chlorpropamide indicates some decrease in favorable results since these earlier observations (TABLE 4). Good or fair control is being obtained in 55 per cent, but some of the patients were those who failed to respond to tolbutamide. A few have improved following administration of chlorpropamide, but the majority fail to improve sufficiently to be classified as successful

TABLE 4
RESULTS OF MAINTENANCE STUDIES WITH CHLORPROPAMIDE* IN 122 DIABETICS

	No of patients	Percentages of total
Good control and fair control	67	55
Insufficient trial	28	23
Primary failures	17	14
Secondary failures	4	3
Side effects	6	5
Total	122	100

* April, 1958, to April 1, 1959

TABLE 5
RESULTS OF MAINTENANCE STUDIES WITH METAHEXAMIDE*

	No of patients	Per cent of total
Good control and fair control†	55	65
Primary failures	19	22
Discontinued—side effects	7	8
Insufficient trial	4	5
Total	85	100

* August, 1958, to March 25, 1959
† After 1 to 8 months

Metahexamide Results obtained with metahexamide from August, 1958, to March, 1959, in 85 diabetics show success comparable to that found with tolbutamide and chlorpropamide (TABLE 5). The rate of primary failure was about 25 per cent, but a number of the patients treated were chosen because of failure to respond to tolbutamide. Of 26 such individuals, 8 promptly failed to respond to metahexamide and 18 were under adequate control A few diabetics responding well to tolbutamide were transferred to metahexamide and obtained equally good blood sugar results with much smaller doses taken once daily.

Toxicity of Sulfonylurea Compounds

Tolbutamide. Tolbutamide administration has been associated with remarkably few untoward effects. Although treatment was discontinued in 9

of the 892 patients reported by Marble because of allergic skin rashes in 6 and
of these upsets
questionable
in 4 patients
jaundice last-
was begun A second
12 months developed a
Postmortem examina-
tion showed mild portal cirrhosis of some duration and a superimposed acute hepatitis, suggesting infectious origin These changes were interpreted* as being not comparable to those seen in a case of liver damage apparently due to carbutamide.

With far better documentation of its relation to tolbutamide, an allergic type of purpura occurred in a 63-year-old man and a 56-year-old woman After taking the drug for 1 and 6 months, respectively, they developed lassi
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No instances of granulocytopenia have been demonstrated Results of

Duration of tolbutamide administration in a large number of patients is now sufficiently long to warrant considerably increased confidence in the drug's safety, since untoward effects requiring cessation of the drug are anticipated in our experience in 1 per cent of the cases or less C J O'Donovan‡ has stated that in more than 9000 diabetics treated with tolbutamide for 6 to more than 28 months, only 1 53 per cent experienced untoward effects requiring omi

Serious or prolonged hypoglycemia can be avoided by

*By William Meissner, Department of Pathology, New England Deaconess Hospital, Boston, Mass

†By James Tullis and Hugh Pyle, Protein Foundation, Boston, Mass.

‡Department of Clinical Investigation, The Upjohn Co, Kalamazoo, Mich.

proper dosage Occasional nausea, vomiting, epigastric distress, generalized weakness, and dizziness occurring singly or together are uncommon since doses of chlorpropamide exceeding 500 mg daily have been discontinued.

Allergic skin reactions have been seen in at least 2 patients, but they were not severe and disappeared promptly on discontinuance of the drug. One man developed jaundice 3 weeks after treatment was started. Tissue removed by needle biopsy of the liver revealed intracanalicular bile stasis and minimal hepatic cellular damage Gradual recovery took place after cessation of drug therapy

No instance of leukopenia, granulocytopenia, or decrease in platelets has been noted to date Liver function tests have yielded results comparable to those found with tolbutamide

Although no cumulative report of chlorpropamide toxicity based on combined experiences is currently available, there were at least 5 instances of jaundice reported at a conference on chlorpropamide and diabetes mellitus in September, 1958 * Some of the cases of toxicity appeared to be related to administration of chlorpropamide in dosages that now seem excessive.

Metahexamide Metahexamide in dosage of 300 mg. per day or higher frequently produces epigastric distress that is usually relieved by reduction in dose However, persistence of these symptoms forced discontinuance of the drug in 4 patients

Three additional diabetics were noted to have abnormal liver function tests while taking metahexamide One woman developed epigastric distress and jaundice during the second week after treatment was started. She received 200 mg daily for 1 week, at which time liver function tests were entirely

developed minimal jaundice and abnormal liver function tests after metahexamide. A third diabetic was found to have abnormal liver function at the time metahexamide was started, but the tests have shown an improvement despite continued administration of the drug

Kirtley reported† that of 1646 diabetics receiving metahexamide as of

Biguanide Compounds

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ticular, with respect to their possible value in replacing insulin in younger and

* Sponsored by The New York Academy of Sciences and Chas Pfizer & Co, Inc.

† At the Conference on Insulin and the Oral Hypoglycemic Agents, Indiana University Medical Center, Indianapolis, Ind, sponsored by Eli Lilly and Company, February 27 to 28, 1959

more severe cases. The brief presentation below of the Joslin Clinic experience with the biguanides when supplemented by the more detailed account by Krall,[26] will indicate the extent to which such optimism necessarily must be limited

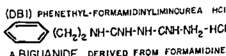
In the United States clinical trials with the biguanides, now conducted more than 2 years, are second in duration only to tolbutamide among the oral agents available Although they have now been given to approximately 5000 diabetics, their clinical use has been in the hands of relatively few investigators and there has not occurred a rapid extension of their administration to many thousands of patients such as that following the introduction of the sulfonylureas

Since both laboratory and clinical data concerning biguanide compounds are based primarily upon the use of the phenethylbiguanide (phenethyl form

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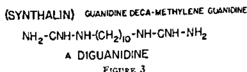


FIGURE 3

normal amyl (DBB)* or normal butyl (DBV)* biguanides The 3 preparations

humans[28] is unexplained

In addition to lowering blood sugar in normal, alloxan diabetic,[23] depin-

liver, (3) absence of hyperglycemic response to epinephrine and glucagon, (4) elevation in the blood of the diabetic of the lactate, pyruvate, and citrate levels, (5) a fall or no change in hepatic vein-glucose output, and (6) the occurrence of gastrointestinal side effects, dosage-related and apparently not of local origin, with anorexia, nausea, vomiting, and diarrhea.

*U S Vitamin Corporation, New York, N Y

The mechanism by which these metabolic effects are produced and blood sugar lowered appears to be that of increased anaerobic glycolysis, occurring as a result of decreased glycolysis via the oxidative pathway of the tricarboxylic acid (Krebs) cycle. Evidence of interference with energy transfer due to a block or partial block in the oxidative pathway has been reported by Wick *et al*[33] and by Steiner and Williams.[34] However, the site of action of the biguanides is still controversial, particularly with regard to (1) the relative contributions of peripheral tissues and liver to the glycolysis induced and (2) the possibility that in diabetic humans, glycolysis via the hexose monophosphate shunt may be increased[35]

Pharmacology Clinical observations suggest that biguanides are rapidly

No other pharmacodynamic effects of DBI have been observed.

Biguanides in the Management of Diabetes

Joslin Clinic patients were first given clinical trials with DBI in December of 1956 The current series, carefully followed and studied under the supervision of Krall, now totals more than 350 individuals. However, such numbers of diabetics do not represent wholesale selection for trial, since they have been carefully culled from approximately 10,000 diabetic admissions to the New England Deaconess Hospital

Blood sugar lowering Early in the clinical trials with DBI it was extremely difficult to demonstrate significant reduction of hyperglycemia comparable to that produced by insulin or, in the responsive patient, by a sulfonylurea. This is accounted for by two observations

(1) The effect on blood sugar is in most instances gradual as dosage is increased, and attempts to give a large loading dose of a biguanide is frequently followed by gastrointestinal side effects.

(2) All patients appear to need some insulin, either endogenous or exogenous in origin, in order to obtain continuing regulation with biguanide compounds

Therefore, when insulin is omitted or significantly reduced in the more severe cases a number of hours before administration of a biguanide, the momentum of the uncontrolled diabetes is sufficient to make the compound ineffectual. In such instances insulin must be given promptly to prevent serious ketoacidosis

Selection of patients Short of clinical trial, no definite clue is available to determine which diabetic patients will do well Diabetics of all ages, durations of diabetes, and previous insulin dosages have been studied. However, during ketoacidosis, infections, surgery, and periods of severe stress, the biguanides almost invariably have been replaced by insulin.

Results. Results with biguanides given for periods as long as 26 months, in studies heavily weighted with severe cases, continue much the same as those in 173 patients previously reported by Krall and Bradley.[27] Blood sugar lowering in the earlier s

significant side effects ar

tinal symptoms of suffic

12 per cent of the patients the side effects occurred so early that dosage sufficient to produce adequate reduction of hyperglycemia could not be tolerated. Nineteen of 31 diabetics classed as failures with sulfonylureas responded without significant side effects.

Maintenance studies. In the 107 diabetics obtaining adequate response to biguanides without side effects, 64 (60 per cent) were rated as having good control by the strict metabolic criteria cited above (TABLE 6). An additional 31 patients (29 per cent) had fair control and the rest rated as poor.

The striking feature in diabetics treated with DBI plus insulin has been stabilization of the diabetes in many individuals, of benefit particularly in the extremely unstable, often long-duration case having severe insulin reactions and threatened with loss of employment. Approximately 30 of these unusually severe cases have been dramatically improved. Despite the maintenance of remarkably good blood and urine sugar values, severe hypoglycemic reactions have been virtually eliminated in these patients.

With biguanides, many patients are managed on insulin doses that are 20

TABLE 6
RESULTS OF MAINTENANCE STUDIES WITH BIGUANIDES

	Number of patients	Percentage of initial successes	Percentage of all patients tried
Good control of hyperglycemia and glycosuria	64	60	37
Fair control of hyperglycemia and glycosuria	31	29	18
Poor control of hyperglycemia and glycosuria	12	11	7
Total	107	100	62

to 50 per cent lower than those used before their administration. In a few instances patients on biguanides alone or with insulin will, after days or weeks or months, develop lethargy, weight loss, and lack of well-being. Addition of insulin or a slight increase in dose rapidly has restored these individuals to normal.

Side effects. Anorexia, nausea, gas, metallic taste in mouth, vomiting, and diarrhea always occur when dosage of biguanide is raised far above hypoglycemic levels. They are classed as side effects because they are always rapidly and completely reversible within 24 hours after omission of DBI. Side effects, if severe, can contribute to the risk of ketoacidosis, so that a supplementary dose of crystalline insulin may be needed. In most instances DBI can be resumed at the meal following manifestation of the side effect or the one following, but in reduced dosage.

Toxicity of the biguanides. In striking contrast to the toxic effects reported to occur in the liver and kidneys following administration of Synthalin is the absence of demonstrable injury to the hematopoietic system, liver, or kidneys of animals[26] or humans[27,28] following biguanide administration. However, Creutzfeldt and Moench[29] after intravenous administration of phenethylbiguanide to guinea pigs in much larger doses than those used orally in earlier

The mechanism by which these metabolic effects are produced and blood sugar lowered appears to be that of increased anaerobic glycolysis, occurring as a result of decreased glycolysis via the oxidative pathway of the tricarboxylic acid (Krebs) cycle Evidence of interference with energy transfer due to a block or partial block in the oxidative pathway has been reported by Wick *et al.*[33] and by Steiner and Williams.[34] However, the site of action of the biguanides is still controversial, [illegible] contributions of peripheral tissue [illegible] the possibility that in diabetic hum [illegible] shunt may be increased[35]

Pharmacology Clinical observations suggest that biguanides are rapidly absorbed, but no data are available giving measurements of these compounds in tissues or blood Measurable blood sugar lowering occurs within 2 to 3 hours in many instances, but duration of action appears to be 8 hours or less. No other pharmacodynamic effects of DBI have been observed.

Biguanides in the Management of Diabetes

Joslin Clinic patients were first given clinical trials with DBI in December of 1956. The current series, carefully followed and studied under the supervision of Krall, now totals more than 350 individuals. However, such numbers of diabetics do not represent wholesale selection for trial, since they have been carefully culled from approximately 10,000 diabetic admissions to the New England Deaconess Hospital.

Blood sugar lowering Early in the clinical trials with DBI it was extremely difficult to demonstrate significant reduction of hyperglycemia comparable to that produced by insulin or, in the responsive patient, by a sulfonylurea. This is accounted for by two observations:

(1) The effect on blood sugar is in most instances gradual as dosage is increased, and attempts to give a large loading dose of a biguanide is frequently followed by gastrointestinal side effects.

[illegible] in [illegible] cases a number of hours before administration of a biguanide, the momentum of the uncontrolled diabetes is sufficient to make the compound ineffectual In such instances insulin must be given promptly to prevent serious ketoacidosis.

Selection of patients Short of clinical trial, no definite clue is available to determine which diabetic patients will do well Diabetics of all ages, durations of diabetes, and previous insulin dosages have been studied. However, during ketoacidosis, infections, surgery, and periods of severe stress, the biguanides almost invariably have been replaced by insulin

Results. Results with biguanides given for periods as long as 26 months, in studies heavily weighted with severe cases, continue much the same as those in 173 patients previously reported by Krall and Bradley[27] Blood sugar lowering in the earlier series was demonstrated in 62 per cent (107) without significant side effects and in 26 per cent with dosages producing gastrointestinal symptoms of sufficient severity to warrant discontinuing the drug In

selected patients both sulfonylurea and biguanide do qualify according to these criteria is the principal reason for their currently expanding role in diabetic treatment.

After having accepted continuing and adequate blood sugar-lowering action as a temporary standard that indicates that oral agents may be antidiabetic in selected patients, the physician should be guided further by the principles outlined below

neuropathy, infections, hepatomegaly, and vascular lesions All diabetics should be trained in the principles of diet and the general measures needed to protect them from acute emergencies

Insulin must be started or dosage increased promptly if blood and urine

an oral agent was added. As time passes and sizable reductions in weight occur, some of these individuals can be taken off the oral agent without a recurrence of hyperglycemia and glycosuria In others, the oral compound must be resumed, usually followed by prompt return to normal of blood and urine sugar values

tained. This has not been our experience

The sulfonylurea response test will eliminate all immediate and most primary

of diabetes.

The incidence of primary failures rises rapidly in those individuals requiring

toxicity studies, have observed certain changes in the liver and kidneys resembling in mild form those found after administration of Synthalin.

Combinations of Blood Sugar-Lowering Agents

Combinations of insulin and tolbutamide have been reported by Fabrykant[40] to be effective in 9 labile and 28 stable but insulin-dependent diabetics. Improved stability was noted in the unstable cases and improved regulation with lowered insulin dosage in those who were stable.

Our experience with either of these techniques is extremely limited. As to

be stimulated. On the other hand a few patients with maturity-onset, stable diabetes responding inadequately to tolbutamide have obtained better blood sugar control when a reduced dose of insulin was added than was produced by insulin alone.

Beaser[41] administered tolbutamide or chlorpropamide with a biguanide to diabetics responding inadequately to a sulfonylurea or biguanide alone. He obtained an additive effect in three and complete replacement of insulin in two patients. A similar technique has been tried in nine of our patients with good control resulting in four, control comparable to that with insulin alone in three, and failure in two.

Discussion

What benefits should oral agents provide? Oral blood sugar-lowering compounds are here to stay for at least the next few years. Not yet available are the truly long-term studies of 10 to 15 years or more needed to evaluate the effects of sulfonylureas and biguanides upon late neurological or vascular complications. Acceptance and use of these agents in diabetics indicates a willingness to undertake the calculated risk that they will not affect victories won for the diabetic[42] through the judicious use of insulin during the past 37 years. Therefore, clearly demonstrable benefits in day to day management of diabetic metabolism in the absence of significant toxicity should offer. (1) better management of diabetes than has been obtainable with diet or with diet and insulin, and (2) demonstrable reduction of hyperglycemia and glycosuria of sufficient degree and duration that control of the diabetes is comparable, and preferably superior, to that obtainable with diet, insulin, or both.

Such stringent criteria are warranted in today's new era of diabetic treat-

ing agents

population

[illegible] tools in treatment. Only by recognizing these limitations and insisting upon good metabolic standards will the physician fulfill his role in protecting his patient from the acute emergencies of diabetes and, in the long run, from late complications. The fact that in

in such matters as diet adherence, adjustment of insulin, and urine tests by all patients Under these conditions, then, blood sugar lowering can be assisted

s, the

DBI

and insulin is justifiable, but such regulation is best carried out in the hospital

Benefits for young diabetics given DBI alone are limited to those in remission where an effort is being made to sustain the remission beyond the usual 3- to 12-month period,[47] and to patients in partial remission in whom 20 U of insulin

insulin appear worthy of further trial in the stable, maturity-onset diabetic, provided that the blood sugar results obtained are superior to those with either

completely responsive to the sulfonylureas

Conclusion

Currently available oral blood sugar–lowering agents are valuable tools capable of supplementing diet and insulin in the total treatment of carefully selected diabetic patients

30 to 40 U. of insulin or more per day. Secondary failures will vary from 5 to 8 per cent and are not clearly related to previous insulin dose. About half are explainable by diet excess or a variety of stressfull situations, and the remainder are idiopathic. The delayed failures are of special importance because of the possible implication that islet-cell exhaustion may be produced through continuing sulfonylurea stimulation. This potential disadvantage of these oral agents has no

In the Joslin

required larger

was resumed. There is, however, a brief period during which higher dosage may be needed to overcome the uncontrolled diabetes.

The roles of chlorpropamide and metahexamide will be determined by the same general principles governing the use of tolbutamide, but are currently modified as follows:

(1) Since they are more potent, chlorpropamide and metahexamide will elicit adequate response in a few of the diabetics inadequately controlled with tolbutamide, however, it is to be expected that some of these patients will become secondary failures.

(2) Longer duration of action allows chlorpropamide and metahexamide to be effective when given in a single dose daily or once in two days.

(3) Offsetting the above two advantages is the question of safety. Although no deaths based on idiosyncratic reaction to these drugs have been proved, their administration has been associated with a disturbing incidence of jaundice, which thus far has been reversible on cessation of chlorpropamide or metahexamide. Concern over the ultimate toxicity of chlorpropamide and metahexamide is not fully eased by the observation that jaundice and other less significant dermatological and gastrointestinal symptoms have occurred primarily when dosage of these agents is excessive.

All sulfonylureas have a maximally effective dosage level beyond which further significant blood sugar-lowering action cannot be obtained.

Despite the limited application of sulfonylureas to selected diabetic patients and the precautions needed to ensure their safety, their blood sugar-lowering action has given the diabetic several striking benefits: (1) reduction of hyperglycemia and glycosuria, apparently attributable in large measure to increased endogenous insulin production and secretion, (2) regulation of carbohydrate metabolism in the fully responsive patient by strict clinical standards, comparable or superior to that obtainable by diet or insulin; (3) control that is often smoother than with insulin and that is associated with a sense of well-being, lessened hunger, and increased incentive to diet adherence, (4) attainment of precise metabolic control with a much reduced risk of serious hypoglycemia in responsive diabetics with occlusive atherosclerotic lesions; and (5)

who are blind or with handi-

and DBB, either alone or in combination with insulin, will lower blood sugar in a large majority (approximately 80 per cent) of diabetic patients. However, gastrointestinal side effects occur with these compounds in one-third of the cases, leaving 50 to 60 per cent in whom they may be considered to be effective. Of this group about

mellitus J. Am Med Assoc 169: 903-909
17 White, P 1956 Natural course and prognosis of juvenile diabetes Diabetes 5. 445-450
18 Duncan, G. G, C T. Lee & J K Young 1957 Clinical experience with the sulfonyl urea compounds Ann N Y Acad Sci 71(1): 233
19 Camerini Davalos, R, H F Root & A Marble 1956 Clinical experience with Orinase A preliminary report. Metabolism 5 904
20 Ibid No 19

glycemic agents Ann. Intern Med 50: 586
28 Fajans, S S, J A Moorehouse, H Doorenbos, L H Louis & J W Conn 1958 Metabolic effects of phenethylformamidinyliminourea (DBI) in normal subjects and diabetic patients Clin Research 6: 252

tern Med 101: 211
31 Ibid No 27

Association, Texas Medical Center, Houston, Texas
36 Ibid No 29
37. Lambert, T. H 1958 Clinical observations with a new oral hypoglycemic agent (DBI) [illegible]

orally. New Engl J Med 259: 1207

Sulfonylurea compounds are effective in the majority of stable diabetics who have an onset age of 40 and over and take 40 U. of insulin or less per day.

Relatively long-term studies with tolbutamide indicate sufficient safety from toxicity and ability to maintain clinical control in responsive diabetics to warrant confidence in its continuing role in management of the disease.

Chlorpropamide and metahexamide are more potent than tolbutamide. They may adequately control some of those patients incompletely controlled by tolbutamide, but their effectiveness in single daily dosage offers an advantage offset by the incompletely resolved question of toxicity. Further cautious clinical trial appears justified.

DBI and its biguanide analogues are capable of lowering blood sugar in many diabetics of all types, but dosage limitations imposed by reversible gastrointestinal side effects restrict their clinical value primarily to stabilization of the extremely severe and labile diabetic. In such patients administration of DBI plus insulin is a particularly refined technique requiring skillful handling

Our experience with biguanides in milder, maturity-onset diabetics is limited. For patients of this group, who are responsive to a sulfonylurea, it has been the preferred oral agent. In those not responsive to a sulfonylurea the possible role of biguanides appears to warrant further trial.

At present no oral agent is preferred for well-controlled, insulin-dependent diabetics free of severe hypoglycemic reactions

AN ASSESSMENT OF ORAL ANTIDIABETIC THERAPY

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The Mount Sinai Hospital, New York, N Y

When tolbutamide became an established form of oral diabetic therapy, it had to measure up to insulin as a standard for therapeutic comparison The generally satisfactory experience with tolbutamide now makes this drug the standard by which all other modifications of the original sulfonylurea formula and unrelated compounds must be measured This paper answers two questions: Do these modifications represent a contribution, and are they safe? In addition an attempt will be made to describe variable responses to the same drug by the same patient.

Variable Drug Responses by the Same Patient

As with insulin, diabetic patients exhibit altering and variable responses to the oral agents according to altering physical stress Primary and secondary tolbutamide failures have been dismissed dogmatically as being insulin deficient in the physiological sense That certain emotional and psychological factors in these patients may alter their responsiveness to these drugs is illustrated by the following cases

FIGURE 1 demonstrates the primary failure of tolbutamide therapy in a 52-year-old woman in whom the daily requirement for 100 U of insulin could not be influenced in the slightest by maximum amounts of tolbutamide while she was followed carefully in the hospital It will be noted that several weeks after the suicide of her husband, when the patient eagerly sought a transfer to tolbutamide and overcame all medical resistance to her request, she was able to effect a successful change from her large insulin dose to 1 gm of tolbutamide, a dose that has been maintained for almost 2 years No attempt has been made as yet to probe into the psychodynamics of this bizarre phenomenon, the only comment the patient has expressed when questioned as to an explanation for the dramatic success has been "God probably took pity on me "

FIGURE 2 illustrates a cyclical variation of responsiveness in a woman whose introduction to diabetes occurred during a severely agitated depression accompanied by ketosis Following a period of insulin therapy an effective change to tolbutamide was accomplished Without explanation a steadily

depressions the psychiatrist had instituted an alarm system whereby any increase of tolbutamide requirement would be reported to him as the sign of oncoming melancholia.

42 eatment of Diabetes

43 Clinical observations
ypamide and diabetes
mellitus, Ann N Y Acad Sci **74**(3): 717.

44 *Ibid* No 17

45 HARWOOD, R 1957 Severe diabetes with remission. New Engl. J. Med. **257**: 257.

46 NEWBURGH, L H & J W CONN 1939 New interpretation of hyperglycemia in obese middle aged persons J Am Med Assoc. **112**: 7.

47 KRALL, L P, P WHITE & R F. BRADLEY. 1958 Clinical use of the biguanides and their role in stabilizing juvenile-type diabetes. Diabetes. **7**: 468–477.

each injection of insulin produced a huge hematoma An attempt to achieve replacement of the insulin with tolbutamide failed at the time With some reluctance DBI was started and doses of 50 mg/day effected a reduction and, finally, abolition of the need for insulin, with excellent control of glycosuria and normal blood sugar levels Soon after he required 100 mg of DBI, then 150 mg, and finally 200 mg. which could not control glycosuria or ketonuria without severe nausea and diarrhea At this time a few days of insulin therapy were effective in obtaining control of the diabetes and a second trial with tolbutamide proved successful Since that time he has been managed with 0 5 gm. of tolbutamide daily.

Such dramatic instances make it evident that considerable fuzziness and overlapping will characterize any attempt to delineate sharply the areas of action and superiority of one drug over another

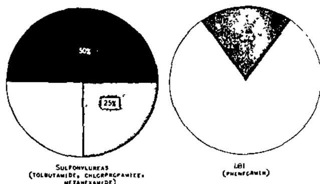


FIGURE 3 Percentage of all diabetics responsive to oral agents

Contribution or Duplication?

[illegible] amide (Orinase*), [illegible] nex‡, Euglycin*) [illegible] are identical, as illustrated in FIGURE 3 The only therapeutic differences lie in the relative increased potency of the newer compounds with effective dosage in the range of one tenth (metahexamide) to one third (chlorpropramide) that of tolbuta-

FIGURE 3. It is apparent that only a fringe of a few per cent may reveal

* Product of The Upjohn Company, Kalamazoo, Mich
† Product of Chas Pfizer & Co, Inc, Brooklyn, N Y
‡ Product of Eli Lilly and Company, Indianapolis, Ind

The first patient represents a primary failure and secondary success. The second patient was a primary success and secondary failure on a cyclical pattern. One wonders whether some of the other primary or secondary failures with oral diabetic therapy may be due to a basic emotional dependency upon

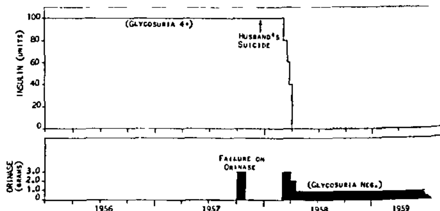


FIGURE 1 Secondary successful response after primary failure. Patient R F, female 52 years, D M, 5 years duration

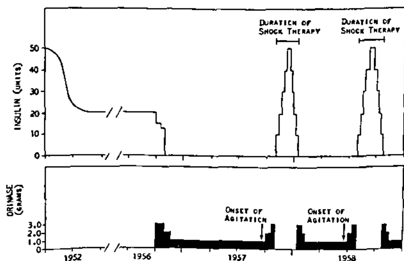


FIGURE 2 Mental depression and the cyclical response to Orinase Patient S W., female, 62 years, D M onset in 1952, ketosis and severe depression

insulin, injections, and the obvious associated implications of a masochistic need.

Variations by the same patient in response to different agents is illustrated

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Furthermore, the contrast between the safety of tolbutamide therapy and the

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iety-producing for both patients and physicians

Side Effects and Toxicity

In May 1957, at a meeting of the Food and Drug Administration at which the public release of tolbutamide was being considered with some hesitancy, Perrin H Long stated that his vast experience with thousands of analogues of sulfonamide type led him to expect no toxic effects when the compound contained a methyl group in the para position of the benzene ring This prediction

TABLE 1

COMPARISON OF SIDE EFFECTS AND TOXICITY OF ALL ORAL AGENTS

Drug	Dosage Range in mg	G I intolerance	Liver damage	Severe hypoglycemia	Acetonuria	Neurological effects
Tolbutamide	500–3000	0	0	0	0	0
Chlorpropamide	250–1000	+	+	+	0	+
Metahexamide	50–250	+	+	0	0	+
DBI (Phenformin)	100–150	++++	0	0	+	0

ates

as-

ceiling of 3-gm. maximum can be exceeded manifold without distress In striking contrast every one of the other three agents has a distinct maximal

succumbing to diabetic ketosis Anorexia, loss of weight, listlessness, and

superior results from one of the newer modifications. Limitations in juvenile diabetes, unstable diabetes, and ketosis that have characterized the early experience of tolbutamide therapy hold true in identical fashion for chlorpropamide and metahexamide Where the former fails the latter two drugs will not succeed so that these newer agents will not reduce the hard core of insulin-deficient patients to any degree.

On the other hand, my own experience indicates that Phenformin is limited in usefulness to less than 20 per cent of the diabetic population. This figure includes a number of mild diabetic patients not requiring insulin in whom asymptomatic glycosuria can respond to a therapeutic, but minimal dosage of DBI. Limitations of therapeutic range will be described in the section of this paper devoted to side effects I have not been able to duplicate the purported claim that DBI will abolish, reduce, or stabilize the insulin requirements in a single insulin-treated patient The most encouraging results with DBI have accompanied its use as a supplement to existing tolbutamide therapy in some primary and most secondary failures. Totally depancreatized humans on DBI require biweekly doses of insulin along with DBI. Keeping this in mind and recognizing the possible synergistic insulinlike effect of tolbutamide in such cases of DBI intolerance, it became apparent that it could be useful generally to employ suboptimal doses of DBI, 50 mg /day for example, with accompanying amounts of tolbutamide of between 1 to 2 gm./day This was begun first with secondary failures on tolbutamide therapy. Secondary failures have been presumed to represent some block by an adaptive mechanism to the action of this sulfonylurea It has been possible in such instances to regain therapeutic success after a temporary period of insulin therapy, or by replacing the tolbutamide with another sulfonylurea derivative. However, with small doses of DBI it was possible to overcome the failure of tolbutamide in about 150 patients who were able to continue with total oral therapy without any side reactions from DBI More recently I have employed the same technique in some primary whom the 3-gm max 4 days in controlling [illegible] It was found that the addition of 50 mg of DBI to the tolbutamide dose very often effectively restored satisfactory control At this point the tolbutamide could be reduced and the DBI discontinued Whenever the tolbutamide was discontinued in this combined therapeutic approach, glycosuria recurred.

Since all side reactions can be avoided, this technique of combination therapy offers the safest method of DBI administration, in addition to providing a solution for tolbutamide-resistant patients.

Of about 1000 diabetic patients attending the outpatient clinic of the Mount Sinai Hospital, about 200 are treated by mild dietary restriction alone, about 400 require insulin, and the remaining 400 use the oral agents In the latter group, 329 patients are well controlled by tolbutamide alone, 50 use a combination

on

on [illegible]

physicians to resort to these more toxic drugs in the face of such overwhelming satisfactory therapeutic success with tolbutamide in the bulk of patients.

CORRELATION OF PLASMA LEVELS OF ORAL ANTIDIABETIC AGENTS WITH BLOOD SUGAR RESPONSES

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Medical Hospital of the University of Freiburg, Freiburg i Br, Germany

With the introduction of sulfonylureas into diabetes therapy 3½ years ago, it could be expected that the substances used in the beginning would not be the most effective In acute experiments upon human beings as well as upon animals, tolbutamide was found to be more effective than carbutamide

only [1,2] Therefore, in long-term therapy carbutamide produces a somewhat better effect. The better effect of chlorpropamide observed by some authors too (36 to 40 hours ly effective plasma per cent)

Occurring for the first time in metahexamide, the relation between plasma level and therapeutic effect seems to be different from that of the sulfonylureas known until the present The necessary therapeutic doses of metahexamide, as well as its plasma levels, are much lower than those of the other sulfonylurea drugs In 10 diabetic persons treated with metahexamide, we found plasma levels of 1 to 4 mg per cent The lower values were found in the morning, the higher values, at noon time.

For an exact comparison of the different efficacies of tolbutamide and of metahexamide, both substances were given orally and intravenously to healthy fasting adults and blood sugars and sulfonylurea plasma levels were determined

In addition, some animal experiments were carried out to test whether there is any sign for a different mode of action of the two substances

Material and Methods

Every 4 to 5 days, 10 healthy, fasting adults were given tolbutamide and metahexamide respectively, in different oral doses (TABLE I) at 8 A M under resting conditions. The blood sugars were determined in the capillary blood

[illegible] meta- [illegible] eter- [illegible] the medicament. In these cases venous blood was taken for the plasma level determinations before the application of the medicaments, and 3, 30, 60, and 240 min and 24 hours, thereafter After oral and intravenous application

* Fellow of the Juan March Foundation, Madrid, Spain
† Supplied by C F. Boehringer and Sons, Mannheim Waldhof, Germany

intermittent acetonuria without glycosuria are further indications of overdosage with DBI

Both metahexamide and chlorpropamide have produced bizarre neurological phenomenon such as asthenia, ataxia, and vertigo in the absence of hypoglycemia. A purported advantage of more prolonged hypoglycemia effects from chlorpropamide has been a disadvantage in my opinion inasmuch as the elderly, senile, arteriosclerotic diabetic patient may suffer irreversible brain injury from such a "beneficial" effect.

No hepatic dysfunction or histological evidence of liver injury has been reported thus far in the 400,000 patients using tolbutamide in the United States On the other hand, a number of cases of liver injury from the other sulfonylurea compounds has been observed. In fact, I learned that of 1800

mg. daily, was instituted for approximately 3 weeks, on the twenty-fourth day mild definite jaundice developed with chemical confirmation of obstructive icterus Liver function studies were otherwise normal, and a biopsy of the liver revealed central lobular necrosis with focal necrosis and mononuclear cell infiltration. This pathological finding resembles the liver injury observed with sulfanilamide in the early days of experience with that drug In contrast to this type of liver damage I have observed two patients with jaundice due to chlorpropamide in which the picture resembles that of cholestasis. Such a case was a 67-year-old woman who had diabetes mellitus for 15 years, requiring about 100 U. of insulin daily Three weeks after she had received chlorpropamide, 250 mg. daily, she developed obstructive jaundice which lasted almost 3 months The biopsy revealed hyperergic cholangiolitis, severe cholestasis (biliary calculi) and focal liver

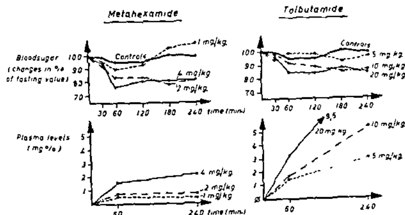
patients receiving metahexamide. It seems that carbutamide was withdrawn for even less frequent toxicity.

In comparing the side effects and toxicity for the newer oral agents (TABLE 1) with the area of population covered by the therapeutic spectrum of FIGURE 3, it should become apparent that the maximum therapeutic effectiveness with maximum safety is to be found in tolbutamide. If serendipity be responsible for the entire field of oral agents in diabetic treatment, the same happy accident has given us the most widely applicable and safest of these agents in the form of tolbutamide.

Acknowledgment

I am indebted to Hans Papper and Fenton Schaffner for interpretation of the biopsy material.

was 28 per cent. An adequate effect could be reached only after 20 mg /kg tolbutamide Four mg /kg metahexamide had a better blood sugar decreasing effect than did 20 mg /kg. of tolbutamide



Mean bloodsugar values and plasma concentrations of metahexamide and of tolbutamide in 10 healthy adults each given three different doses orally

FIGURE 1

are 5 times higher than in metahexamide The correlation is not very good in the lower metahexamide doses, it results from the difficulty of an exact determination of plasma levels below 1 mg per cent

but 30 min. after application of metahexamide the blood-sugar decrease amounts to 35 per cent. The plasma levels of the two substances, however, differ by 20 per cent only It is difficult to say whether this is due to the method or to a larger distribution space or to a different kind of protein bind-

of 4 mg/kg metahexamide, urine was collected from 5 persons for a determination of the amount of metahexamide at the same time when blood was taken for the determination of the plasma levels. Finally, 5 persons were given 6 mg/kg metahexamide intravenously, and the plasma levels of metahexamide were determined *30 min*, and *3, 7, 10,* and *24 hours thereafter.*

Tolbutamide plasma levels were determined by the method of Spingler[9]

The determ

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of metahexamide was in aqueous solution 96 ± 3 per cent, in plasma 53 ± 1.8 per cent. As in different metahexamide concentrations, the same plasma recovery percentage was found, all measured plasma values were doubled. All plasma level figures were determined by this type of calculation.

TABLE 1

Dosage (mg/kg, orally)	Blood sugar decrease in percentages			
	Metahexamide		Tolbutamide	
	After 60 min	Maximum	After 60 min.	Maximum
1	12 ± 13 6	14 ± 11 8		
2	16 ± 13 5	28 ± 8 3		
4	24 ± 9 2	30 ± 8 7		
5			1 ± 6 2	15 ± 5 8
10			8 ± 13 0	18 ± 4 2
20			15 ± 5.5	22 ± 3.4
Fasting controls	6 ± 6 8	10 ± 3 8		

(Mean values in 10 healthy adults)

Animal experiments Eighty albino rats from an inbred stock with a body weight of *250 to 300 gm* were given a standard cake diet, *22 of these animals* served as controls Fifty-eight animals were given 50 mg/kg. metahexamide by an esophagus tube for different periods of time On 14 rats, blood was taken from the tail vein before the administration of the medicament and 3 hours thereafter. The animals were sacrificed by a blow on the neck 3 and 24 hours, respectively, after a single application of metahexamide, or on the fifth and fifteenth day, respectively, if metahexamide was given daily. The pancreas of each animal was fixed in Bouin's solution, and paraffin sections stained with Azan in the modification of Gomori[11] and with aldehyde-fuchsin phloxin by Maske.[12] From 53 animals a piece of the right median liver lobe was removed immediately after the killing, weighed on the torsion balance, and the liver glycogen determined by the anthrone method of Seifter *et al*[13]

Results

(1) *Blood sugar decreases, plasma levels, and half-lifetime in healthy adults* TABLE 1 shows that the maximum blood sugar decrease in the control test was 10 per cent The oral administration of 1 mg/kg. metahexamide and of 5

(2) *Beta-cell degranulation and liver glycogen in rats after administration of metahexamide.* As metahexamide, according to these results, is considerably more effective than the sulfonylureas used until now, the question may be asked



... staining as in 2)

if this substance has the same mode of action as the others. Among other things, after application of sulfonylureas an impressive degranulation of the

ing On performing dialysis experiments, we found a firm protein binding of metahexamide Paper electrophoretic investigations that have not been finished as yet show no self-movement of tolbutamide in sodium diethyl barbituric acid but an elective movement with the albumin, in contrast to metahexamide and carbutamide that show self-movement. Furthermore, on the elution after serum electrophoresis larger amounts of metahexamide and of carbutamide are found in the albumin band.

FIGURE 3 shows electrophoresis strips of tolbutamide, of carbutamide, and of metahexamide for comparison. At the bottom of each pattern there is a

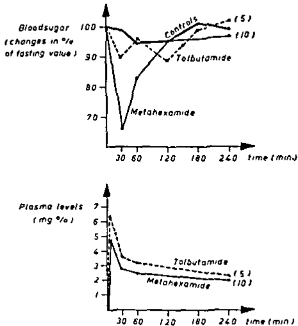


FIGURE 2 Mean blood sugar values and plasma concentrations of metahexamide and of tolbutamide in 10 and 5 healthy adults respectively, each given 4 mg/kg intravenously

strip with sulfonylurea only, beyond this another one with sulfonylurea in serum, and on the top the same strip as in the middle after protein staining

Within 24 hours after intravenous application of 280 mg, about 70 per cent of the administered metahexamide was found in the urine (5 persons excreted

tion the half-lifetime of metahexamide is, based on our own observations only

ml plasma/min

[illegible] As in our present exami-

TABLE 3

Variations of liver glycogen in rats

	after	
	Tolbutamide (1956) (250 mg/kg, orally) (percentage)	Metahexamide (1959) (50 mg/kg, orally) (percentage)
3 hrs. after one dose (starved for 24 hours)	+400 ($p < 0.001$)	+230 ($p < 0.001$)
Treated 5 days, last dose 3 hrs before death (starved for 12 hours)	+250 ($p < 0.02$)	−18 (n s)
Treated 14 days, last dose 24 hrs before death (starved for 12 hours)	+630 ($p < 0.001$)	−7 (n s)

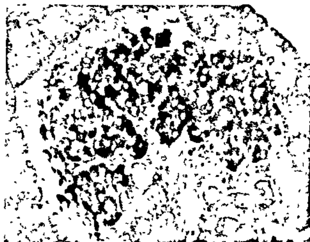


FIGURE 5. Pancreatic islet of a normal rat (fasting control) Bouin's, paraffin section stained with aldehyde fuchsin phloxin [18]

FIGURE 4 shows in the left part that there is a significant increase of the liver glycogen in fasting rats 3 hours after application of metahexamide. At the same time, the blood sugar decrease caused by the dose of 50 mg /kg is 57 ±

TABLE 2
RENAL EXCRETION OF METAHEXAMIDE AFTER A SINGLE ORAL APPLICATION OF 300 MG (HEALTHY ADULTS)

Case No	Mg (0 to 24 hrs)	Mg (24 to 48 hrs)
1	234	45
2	217	65
3	218	72
4	164	85
5	191	77
6	266	30
7	204	53
8	181	40
9	279	23
Average (mg)	217	54
Percentages of intake	72	18

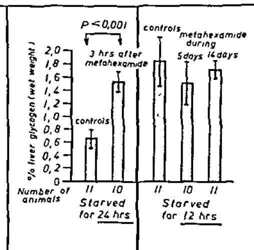


FIGURE 4

3.2 per cent Thus far there is no difference between this action and that of the other sulfonylureas In addition, increased liver-glycogen values had been found after a continued application of tolbutamide if the dose was not higher than 250 mg /kg body weight.[19 21] This effect is not demonstrable after application of metahexamide, as follows from the right part of FIGURE 4

some time there is an adaptation to the treatment with sulfonylureas, so that a moderate regranulation begins despite the continued medication [14] FIGURE 8 shows a pancreatic islet of a rat after 14 days of uninterrupted metahexamide medication The beta cells contain some granules again

In the alpha cells no changes were noticed

Summary

Our investigations showed that metahexamide is at least 5 times more effective in healthy adults than is tolbutamide The therapeutically effective plasma level of metahexamide is accordingly only 20 per cent of that needed by tolbutamide This stronger effect of metahexamide is not due to a pro-



FIGURE 8 Pancreatic islet of a rat after oral application of 50 mg /kg metahexamide for 14 days Slight regranulation of the beta cells (stained as in FIGURE 5)

hexamide application a beginning degranulation of the beta cells becomes visible After 24 hours it already is very pronounced FIGURE 5 shows the normal beta-cell granulation of a pancreatic islet in the rat. FIGURE 6 demonstrates the heavy degranulation of beta cells 24 hours after oral application of 50 mg /kg metahexamide, and FIGURE 7 the typical picture of completely degranulated beta cells in the pancreatic islets of rats treated for 5 days After



FIGURE 6 Pancreatic islet of a rat, 24 hours after one oral dose of 50 mg /kg. metahexamide Significant degranulation of the beta cells (stained as in FIGURE 5)

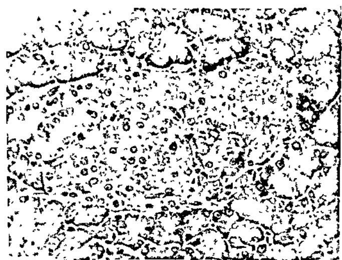


FIGURE 7 Pancreatic islet of a rat after oral application of 50 mg /kg metahexamide for 5 days Complete degranulation of the beta cells (stained as in FIGURE 5)

COMPARATIVE PHARMACOLOGY AND CLINICAL RESPONSES TO METAHEXAMIDE*

George J Hamwi, Thomas G Skillman, Fred A Kruger, William H Roush, Lucy R Freedy†

Department of Medicine, The Ohio State University, Columbus, Ohio

Metahexamide (N-(3 amino-4-methyl benzine-sulfonyl)-N'-cyclohexylurea) was studied for the purpose of comparing its hypoglycemic potency, toxicity, and pharmacology with the currently existing compounds used for oral therapy of diabetes

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In addition, normoglycemia is obtained in responsive patients with serum concentrations of metahexamide that range from one fifth

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of about 10 per cent. This incidence is comparable to that of chlorpropamide. However, preliminary data suggest that alterations of liver function are more frequent with metahexamide than with other compounds

Methods

mide were chosen deliberately All patients were maturity-onset diabetics

mg per cent were regarded as responsive while those displaying values above this critical level were considered unresponsive Control values of liver function, renal function, and hematopoietic function were established by measurement of cephalin flocculation, serum bilirubin, thymol turbidity, albumin, globulin, cholesterol, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGO-T), Bromsulphalein (BSP) retention, hematocrit, white cell

rantee of the National Institute of Arthritis and Metabolic Diseases, Bethesda, Md

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Metahexamide (N-(3 amino-4-methyl benzine-sulfonyl)-N[1]-cyclohexylurea) was studied for the purpose of comparing its hypoglycemic potency, toxicity, and pharmacology with the currently existing compounds used for oral therapy of diabetes

Observations made during a 6-month period in which metahexamide was given in daily doses ranging from 50 to 500 mg./day to 73 maturity-onset diabetic patients permit the conclusion that this compound embodies a hypoglycemic potency that is approximately 7 times that of tolbutamide and twice that of chlorpropamide In addition, normoglycemia is obtained in responsive patients with serum concentrations of metahexamide that range from one fifth to one third as high as the levels needed with the other sulfonylureas Early studies of the incidence of untoward reactions to metahexamide indicate an incidence of about 10 per cent This incidence is comparable to that of chlorpropamide However, preliminary data suggest that alterations of liver function are more frequent with metahexamide than with other compounds

METHODS

mide were chosen deliberately All patients were maturity-onset diabetics

...el glucose values

Metahexamide

... periods of 15 to

180 days Insulin was discontinued at the onset of therapy in patients who were using it, and in all cases the usual diet was continued In the patients who had been managed with chlorpropamide, metahexamide was substituted in the same daily dosage where feasible, and the average blood glucose levels obtained during therapy with each agent were compared Patients who consistently maintained fasting and postprandial blood glucose levels below 150 mg per cent were regarded as responsive while those displaying values above this critical level were considered unresponsive Control values of liver function, renal function, and hematopoietic function were established by measurement of cephalin flocculation, serum bilirubin, thymol turbidity, albumin, globulin, cholesterol, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGO-T), Bromsulphalein (BSP) retention, hematocrit, white cell

counts, differential counts, prothrombin times, blood urea nitrogen (BUN), creatinine, and uric acid concentrations. Other studies included protein-bound iodine, thyroidal radioactive iodine uptake, and urinary excretion of 17-ketosteroids and 17-hydroxycorticoids. In most patients blood glucose and serum metahexamide levels[1,2] were obtained at weekly intervals, and the other indices were obtained frequently during the period of therapy with metahexamide

Results and Discussion

Pharmacological Studies

Dosage Favorable hypoglycemic effects were seen with daily doses ranging from as low as 50 mg /day to as high as 250 mg /day. The average dose of

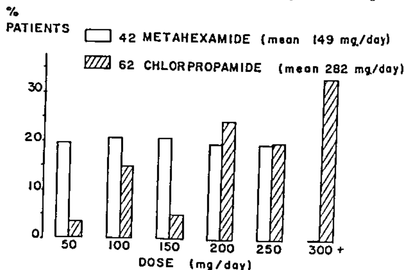


FIGURE 1 Daily maintenance doses of metahexamide with chlorpropamide in responsive patients

metahexamide on the day that normoglycemic levels were achieved in the first 30 responsive patients was 235 mg./day. The dose needed to preserve such

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In the

42 patients responsive to metahexamide it was necessary to employ 5 different

mg /day. In contrast,

77 per cent of the 62 chlorpropamide-responsive patients required daily maintenance doses of 200 mg or more and averaged 282 mg /day, which is approximately twice the amount needed with metahexamide. A similar decrease in maintenance requirement with chlorpropamide was observed as the dosage fell from 435 mg /day at the time of initial response to 282 mg /day This tend-

ency for the magnitude of dosage to decrease during maintenance is of clinical

Striking variations of levels were found in individuals receiving the same daily doses of metahexamide, but in general a rough correlation between the daily dose and the serum levels was noted (FIGURE 2) For example, patients taking

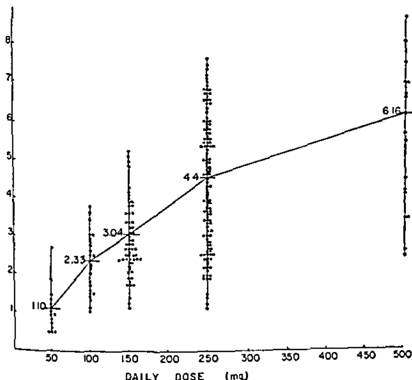


FIGURE 2 Plasma metahexamide levels (milligrams per cent).

averaged
o plateau
mg daily

also be noted that maximum serum levels are achieved only after 6 to 8 days of administration of a constant daily dose The observed increment in drug concentration during initial therapy is probably best explained by the relatively

long half lives of these compounds. It has been shown previously that the biological half life for chlorpropamide averages 34 hours.[3,4] Our studies indicate that serum concentrations of metahexamide fell 50 per cent within 17.5 to 22 hours (average 19 hours) following the oral ingestion of this compound (FIGURE 4) It is of further interest that, in the 4 individuals in whom the half-life study was done, calculations of a theoretical "metahexamide space" averaged 12 l, and in no patient was this thought to be in excess of extracellular fluid volume

To demonstrate the differences in serum levels attained in the same patient on the same dosage of
was followed for 2 mont
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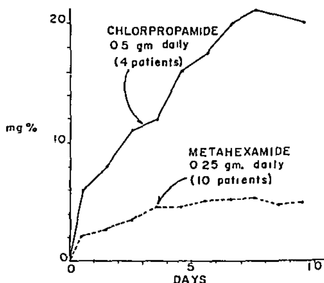


FIGURE 3 Blood levels achieved with maximum daily maintenance doses

drugs were alternated, so that each patient who had received metahexamide was placed on chlorpropamide in the same dose, and vice versa. FIGURE 5 demonstrates that on the same dose the metahexamide serum levels are distinctly lower than the serum levels obtained on chlorpropamide The explanation for this difference in serum levels of these 2 compounds is still doubtful. It is clear, however, that the effective serum levels of metahexamide are one fifth to one half of the serum levels obtained with chlorpropamide

Hypoglycemic effect Forty-four of the 73 patients (60 per cent) responded to metahexamide in a satisfactory fashion In each instance blood glucose values fell from hyperglycemic levels to fasting and postprandial levels which remained below 150 mg per cent during the entire period of therapy The mean
150 mg
level of

ranging from 1.4 to 5.8 mg. per cent were observed. Some of the characteristics of the responsive and nonresponsive patients are listed in TABLE 1. It may be noted that the responsive patients as a group had been taking less

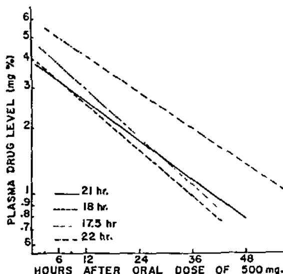


FIGURE 4. Plasma half life of metahexamide.

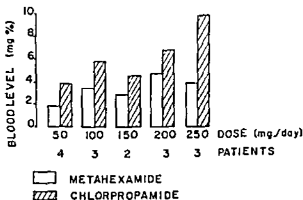


FIGURE 5. Differences in blood levels at various dosages.

insulin and reported a shorter duration of diabetes than did the nonresponsive patients.

Standard oral glucose tolerance tests were performed before and after 1 week of metahexamide therapy in 8 responsive patients. As demonstrated in FIGURE 7, no essential change in the configuration of the curve occurred.

TABLE 1
CHARACTERISTICS OF PATIENTS*

Factor	Responsive (44)	Nonresponsive (29)
Age (years)	61	61
Per cent, women	55	83
Daily insulin (U)	11	22
Years of diabetes	4 6	7
History of acidosis (%)	0	14 0
Dose (mg /day)	150	250
Mean blood level	3 04	5 48
Per cent fall in blood sugar	53 0	13.7

* Figures given as mean

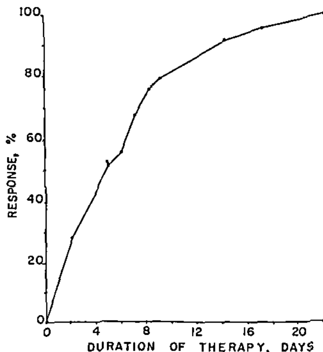


FIGURE 6 Frequency of delay in response to metahexamide in 25 patients

Although the mechanism of this effect is not clear, this is not the response that one would predict from the administration of insulin.

Comparative hypoglycemic effect There were 53 instances in which the response to metahexamide could be compared to that with tolbutamide and chlorpropamide in the same patients. Metahexamide was given to 20 indi-

viduals who previously had been treated with tolbutamide Seventeen of these subjects were deliberately selected because they had been shown to be unresponsive to tolbutamide. Six of the 17 were found to respond to metahexamide All of the tolbutamide-responsive patients responded to metahexamide. This finding indicates that metahexamide may prove useful in tolbutamide-resistant patients

Comparative observations were made in 33 patients who had undergone trials with both chlorpropamide and metahexamide On the basis of response, these patients could be divided into 3 groups (TABLE 2)

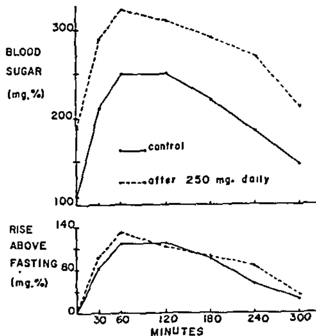


FIGURE 7. The influence of metahexamide on oral glucose tolerance (means of 8 patients)

Seventeen individuals (51 per cent) were responsive to both compounds (Group 1), and 3 patients (9 per cent) proved sensitive to chlorpropamide but not metahexamide, Group 2 Thirteen individuals (40 per cent) failed to respond to either compound No patient was found to be responsive to metahexamide but resistant to chlorpropamide From these observations one might postulate that chlorpropamide may occasionally be effective in the metahexamide-resistant patient It may be of interest to note that in 5 of

enformin*

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* Product of U S Vitamin and Pharmaceutical Corporation, New York, N Y

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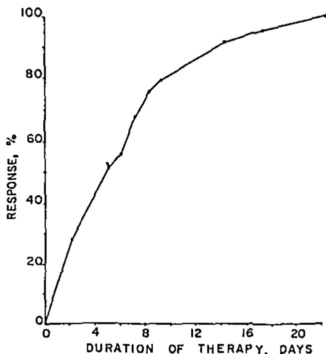


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Other Effects

Endocrine effects. Studies of patients with acute and chronic conditions suggest that metahexamide has no important effect on thyroid or adrenal function

TABLE 3
ENDOCRINE FUNCTION STUDIES

	Test	Control*	Therapy*	Days interval*	No patients
Thyroid	24 hr I^{131} uptake (17 to 37%)	20 (11 to 37)	18 (7 to 33)	56 (7 to 105)	25
	Serum PBI (3.5 to 8 µg %)	5 1 (2 95 to 8 72)	5 2 (2 9 to 7 78)	64 (7 to 120)	34
	Serum cholesterol (130 to 280 mg %)	238 (113 to 400)	225 (150 to 395)	64 (7 to 120)	43
Adrenal	17 OH/24 hr (5 to 15 mg)	8 6 (0 5 to 23 8)	12 4 (1 1 to 14 0)	5 (1 to 9)	12
	17 ketosteroids/24 hr (5 to 20)	6 26 (5 6 to 30 1)	6 65 (1 1 to 14 8)	5 (1 to 8)	11

* Mean and range

TABLE 4
HEMATAPOIETIC AND RENAL FUNCTION TESTS

Tests	Control*	Therapy*	No patients	Days interval*
Hb	13 2 gm % (10 5 to 15 9)	12 4 (8 0 to 15 8)	41	71 (7 to 135)
Hct	42 5% (35 to 51%)	40 0% (30 to 53%)	29	71 (7 to 135)
WBC	8,382/mm³ (3500 to 22,400)	7,086 (4500 to 12,850)	54	63 (7 to 120)
Platelet count	213,124/mm³ (34,000 to 728,000)	214,933 (116,000 to 424,000)	14	10 (6 to 14)
Bleeding time	1'23" (45"–2'0")	2'5" (55" to 6'0")	9	73 (4 to 150)
Clotting time (cap)	6'0" (3'25" to 7'35")	3'10" (1'4" to 7'15")	7	73 (4 to 150)
BUN	18 mg % (9 to 44)	18 (10 to 45)	48	71 (7 to 135)
Creatinine	1 6 mg % (1 0 to 2 7)	1 8 (1 0 to 3 4)	14	71 (7 to 135)
Uric acid	3 5 mg % (2 7 to 5 9)	3 2 (0 8 to 7 4)	13	71 (7 to 135)
PSP	15 min 30% 2 hr 67%	15 min 20 25% 2 hr 43 75%	2 2	0 (7 to 10)

* Mean and range in each instance

Values of these indices of endocrine function obtained before and during treat-

TABLE 2
COMPARISON OF DOSAGE AND BLOOD SUGAR LEVELS IN 33 DIABETIC PATIENTS TRANSFERRED FROM CHLORPROPAMIDE TO METAHEXAMIDE

	Chlorpropamide trial period			Metahexamide trial period		
	FBS (mg %)*	Daily dose chlorpropamide (gm)*	Serum level (mg %)*	FBS (mg %)*	Daily dose metahexamide (gm)*	Serum level (mg %)*
Group 1. 17 patients responding optimally to both agents	113 (80 to 150)	0 287 (0 05 to 1 0)	11 1 (3.5 to 23.0)	97.8 (70 to 150)	0 176 (0 05 to 0 50)	3 4 (1 0 to 6 9)
Group 2 3 patients responding optimally to chlorpropamide only	135 (110 to 150)	0 267 (0 10 to 0 50)	12 1 (5 6 to 23 0)	213 (180 to 240)	0.167 (0.10 to 0.25)	3 8 (2.9 to 5 4)
Group 3 13 patients responding to neither agent	248 (180 to 400)	0 438 (0 20 to 1 0)	16 9 (8.5 to 28 0)	237 (165 to 300)	0 254 (0 15 to 0.40)	5.5 (3.1 to 9.8)

* Figures given as mean and range

TABLE 5
LIVER FUNCTION TESTS

Tests	Control*	Therapy*	No patients	Days interval*
TP A/G	7 6 3 4/3 3 mg % (6 2 to 8 8) (3 7 to 5 4)/(2 6 to 4 1)	7 8 4 2/3 1 (6 4 to 8 4) (2 7 to 4 9)/(2 2 to 4 3)	29	79 (7 to 150)
Transaminase	14 7 units (4 to 21)	22 5 (13 to 46)	10	69 (3 to 135)
Bilirubin	T 0 45 mg % (T 0 1 to 0 8)	T 0 3 (T 0 1 to 0 4)	14	71 (7 to 135)
Cephalin floc	neg (neg to 3+)	neg (neg to 2+)		82 (14 to 150)
Thymol turb	10 units (neg –20)	10 (neg –30)	37	79 (7 to 150)
Alk phos	5 8 SJR units (3 6 to 9 2)	6 4 (2 6 to 20 7)	35	70 (4 to 135)
BSP	7% (0 to 21%)	4 5% (2 to 11)	4	7 (4 to 10)
Prothrombin time	78% (50 5 to 100%)	76 8% (45 1 to 100)	21	70 (5 to 135)

* Mean and range in each instance

instance of hemolytic anemia believed to be induced by metahexamide was observed and is discussed below.

Effects on liver function. Values pertaining to several liver functions were determined over periods of observation ranging from 14 to 180 days (TABLE 5). Sporadic elevations of thymol turbidity (2 patients) serum alkaline phosphatase (2 patients), and SGO-T (1 patient), usually of minor proportion, were observed In these patients such deviations were transient and were seen in patients with a definite history of alcoholism or cholelithiasis. In one instance, a causal relationship of derangement of liver function seemed clearly related to

treated with metahexamide 250 mg. daily for 15 days, she was admitted to the hospital with jaundice and dia
mission showed chemical evidence o
rose from normal values to 16 mg
phosphatase rose from 3.5 to 36 8 S
and the SGO-T rose to 263 units. During the period of jaundice the hemoglobin fell from 11 8 gm per cent to 7 3 gm per cent and the reticulocyte count rose to as high as 9 2 per cent. A study of RBC survival using Cr^{51}-tagged cells indicated a slight decrease in RBC survival time. Four serial punch biopsies of the liver showed transient changes characterized by mild focal hepatocellular necrosis, minimal periportal round cell infiltration, and inspissation of bile in the biliary canaliculi The red blood cell content of glucose-6-phosphate dehydrogenase was found to be reduced. These findings are interpreted as consistent with metahexamide-induced cholestasis with jaundice and metahexamide-induced hemolytic anemia in a patient with a congenital deficiency in glucose-6-phosphate dehydrogenase. Continued observation of this patient during the 3 months since the onset of her jaundice indicates that there is no residual evidence of hepatic damage

Side reactions Untoward effects believed attributable to metahexamide were observed in 7 of the 73 patients (10 per cent)

Four individuals developed epigastric distress that occurred after ingestion

of the upper gastrointestinal tract in 2 of these patients showed no evidence of ulcer, gastritis, or abnormal peristalsis.

One patient developed a generalized maculopapular skin eruption on the thirty-first day of therapy on a 250 mg./day dose. The rash was quite extensive but cleared completely within 7 days after the drug was discontinued. A second patient developed rather severe generalized pruritis that was not associated with an eruption This symptom also regressed promptly after therapy was terminated.

Although variations in liver function studies occurred in 6 patients, in only 1 instance could such changes be clearly related to metahexamide administration, this instance of jaundice with hemolytic anemia is described above. It might

3 Johnson, P, A Hennes & K. M West 1959 Metabolic fate of chlorpropamide in man Ann N Y Acad Sci 74(3): 459
4 Knauff, R E, S S Fajans, E Ramirez & J W Conn 1959 Metabolic studies of chlorpropamide in normal men and diabetic subjects Ann N Y Acad Sci 74(3): 57-81

be postulated that in some instances metahexamide may induce changes in liver function that are transient and do not necessarily indicate important toxic manifestations. It will be necessary to observe a large number of patients treated for chronic conditions in order to be able to interpret the significance of such transient elevations of serum alkaline phosphatase, thymol turbidity, or SGO-T, particularly in a group of patients with a potentially high incidence of nondrug-related liver damage.

Summary and Conclusions

respectively.

(3) Normoglycemia was achieved with metahexamide in responsive patients with blood levels ranging from 1.4 to 5.8 mg., and averaged 3.9 mg. per cent. This level is 20 to 33 per cent as high as levels reported as needed with all other sulfonylureas.

(4) The biological half life of metahexamide averaged 19 hours as compared with the average half lives of 34 hours and 4 hours reported for chlorpropamide and tolbutamide, respectively.

(5) Although serum levels within the therapeutic range may be achieved within 4 hours, normoglycemia still had not been achieved after 7 days in 40 per cent of the patients who ultimately responded.

(6) Metahexamide was effective in 6 of 17 tolbutamide-resistant patients and in all of 3 tolbutamide-responsive patients. Metahexamide was effective in 17 of 20 patients who were responsive to chlorpropamide. In no patient resistant to chlorpropamide was a favorable response obtained with metahexamide.

(7) This compound produced no changes in thyroid, adrenal, cardiac, or renal function.

(8) In a total of 73 patients exposed to metahexamide there were 4 instances of gastrointestinal intolerance, 1 instance of generalized maculopapular eruption of the skin, 1 instance of generalized pruritis without eruption, and 1 instance of combined toxic hepatitis and hemolytic anemia. In addition, there were 5 instances of sporadic transient abnormalities in liver function tests, the significance of which is obscure.

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sistant patient.

References

1

Materials and Methods

Animal Experiments

Experiments were performed upon 7 mongrel dogs weighing between 15 and 20 kg each. Animals were maintained on routine laboratory diets and fasted overnight prior to study. The experimental procedure was performed under Pentothal anesthesia. The abdomen was opened and splenectomy was performed. Following this, a No. 60 polyethylene catheter was inserted retrograde into the pancreatic vein. Blood was collected in heparinized tubes at ap-

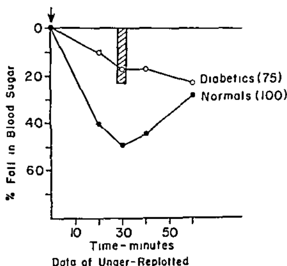


FIGURE 1. Composite curves showing the normal and diabetic blood sugar response to tolbutamide.[4]

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hemidiaphragms obtained from 3 rats were pooled, weighed, and placed in paired Dubnoff flasks. One flask contained 2 ml. of the control plasma-buffer

TOLBUTAMIDE STUDIES IN PREDIABETES*

Henrique Paulo Barros Barreto† and Lillian Recant
Washington University School of Medicine, St. Louis, Mo.

Introduction

It has been shown that the oral hypoglycemic agents of the sulfonylurea type produce hypoglycemia by a mechanism that requires the presence of the beta cells of the pancreas[1] Evidence of many types has accumulated confirming this observation, indicating that insulin is released from the pancreas as a result of the action of these drugs. The evidence for insulin release includes the

responsiveness to the drugs to the adult rather than the juvenile onset type of diabetes

If, indeed, this is the mechanism of action of these agents, it seems reasonable to conclude that the magnitude and type of blood-sugar response to these agents must be dependent upon the quantity of *available* insulin in the pancreas. In this connection, Unger and Madison recently published observations of 75 mild diabetics and 100 normal subjects studied after the administration of 1 0 gm of tolbutamide intravenously[4] A striking difference was noted in the response of the diabetic Not only was the 30-min. fall in blood sugar reduced, namely 82 per cent of cases showed a blood sugar fall of less than 23 per cent of the initial blood sugar as contrasted with 99 per cent of the normals who showed a fall greater than 23 per cent, but the shape of the curve was quite different The diabetic response was characterized by a gradual but continuously falling blood-sugar curve even at 90 min., while the normal response was a rapid fall within 30 min and a rapid return toward the starting value (FIGURE 1).

It is not surprising that in the diabetic the decline in blood sugar is of smaller magnitude. The continuous fall in sugar, on the other hand, suggests that in the diabetic, differences may exist that relate not only to the insulin content of the pancreas but also to the rate of insulin release In an effort to gain some understanding of the nature of the normal and diabetic curve, studies were made of the tolbutamide response of normal dogs and normal human subjects to intravenous tolbutamide The latter was investigated under conditions expected to influence the state of carbohydrate tolerance. During the course of these studies it was noted that certain subjects could be converted from a normal response to that entirely characteristic of the diabetic. These subjects, using the terminology of Conn,[5] have been designated as prediabetics.

* The work reported in this paper was supported in part by grants from The Upjohn Company, Kalamazoo, Mich , and Merck Sharp & Dohme, West Point, Pa.
† Rockefeller Foundation Fellow.

in vitro, using zero-time pancreatic plasma as a vehicle had no effect on glucose release from liver slices, it seemed likely that this effect of pancreatic plasma following tolbutamide was not due to the increased insulin activity but to a

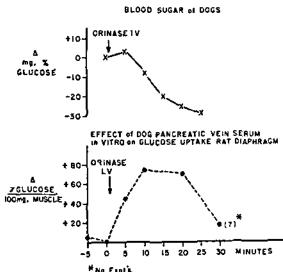


FIGURE 2 Assay for insulinlike activity in dog pancreatic venous blood following tolbutamide

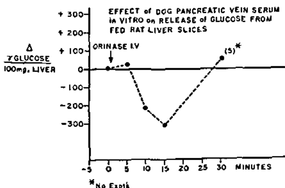


FIGURE 3 Assay for hyperglycemic activity of dog pancreatic venous blood following tolbutamide

solution, while the second flask contained the 10-, 20-, or 30-min samples Plasma samples were run in triplicate. In this way data could be expressed as the change in glucose uptake of the diaphragm incubated in the 10, 20, and 30-min samples following tolbutamide as compared with the 0-min. sample. Insulin activity was then expressed as the change in micrograms of glucose taken up per 100 mg diaphragm per hour. These values were not calculated back to the undiluted plasma The pancreatic samples were also tested for hyperglycemic activity Plasma samples were diluted 1:3 in Ringer-phosphate solution without added glucose Liver slices weighing approximately 200 mg were obtained from fed rats and were incubated in 2 ml of diluted plasma at 37° C in the presence of oxygen for 1 hour. At the completion of the experiment, measurements of glucose appearing in the medium were made The glucose release (μg glucose/100 mg. liver/hour) of the zero time sample was compared with quadruplicate samples obtained at 10, 20 and 30 min. after tolbutamide, the activity was expressed as this change. In a few instances peripheral samples of plasma were assayed for both insulin and hyperglycemic activity

Human Experiments

A group
studied
The group
history of diabetes
night prior to testing
administered intraver
conditions Blood samples were obtained every 15 min. for 1 hour and then at 90 min. Sugars were done in duplicate. The nonesterified fatty acids were measured by the method of Dole[8] Steroids, when used, were Decadron in total dosage of 6 mg taken orally 8½ and 2 hours prior to testing and, in a few cases, 40 mg of Prednisone was used

Results

Animal Experiments

Insulin activity following I.V. tolbutamide. The mean value of glucose uptake by diaphragm incubated in zero-time pancreatic plasma was 320 μg glucose/100 mg wet diaphragm/hour The mean changes observed in pancreatic plasma compared with the zero-time plasma value in the 7 dogs tested may be seen in FIGURE 2 Correlation with the changes in blood sugar also may be seen It may be noted that the maximal change was an increment in the 10 to 20 min sample following tolbutamide, with a fall noted thereafter These changes are significant by analysis of paired data (p = 0 01) No change

ble changes were noted in peripheral blood. Since glucagon-free insulin tested

no change in the curve can be seen Similarly, performance of the test after 24 hours of excessive carbohydrate intake and 2 hours after a high carbohydrate meal produced no alteration in the response. However, in 2 subjects in whom carbohydrate was eliminated for a period of 3 to 7 days and who were maintained on isocaloric diets, high in protein and fat, 2 different responses were noted (FIGURE 5). Subject H B showed a response which was entirely normal. Carbohydrate deprivation produced a change in response in subject M K, who, incidentally, has a very extensive family history of diabetes Prior to carbohydrate deprivation, the time of maximal blood sugar fall following tolbutamide was 45 min with a fall at 30 min of 31 5 per cent Following deprivation, the time of maximal fall was 60 min, and the fall at 30 minutes was 25 5 per cent This delay in time of maximal fall in blood sugar following carbohydrate restriction, approaches the diabetic curve in which the time of maximal fall is delayed beyond 90 min

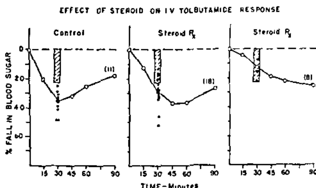


FIGURE 6 Composite curves with the spread of the 30-min values indicated The bar indicates the 23 per cent level of blood sugar fall

Effect of Steroids on I V Tolbutamide Response

The second type of response, termed abnormal, was shown by 8 subjects, it

there is a rapid increase in insulin activity in pancreatic vein blood. This activity does not appear to be sustained, although observations were not made over longer periods than 30 min. In addition, as insulin is secreted, there appears to be a decrease in secretion of hyperglycemic factor.

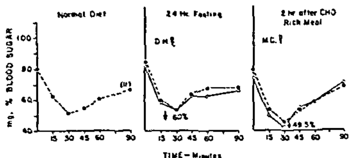


FIGURE 4 Influence of diet on tolbutamide response.

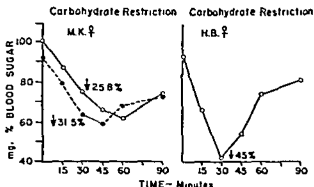


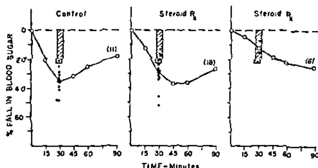
FIGURE 5 Influence of carbohydrate deprivation on tolbutamide response.

Human Experiments

Effect of diet on I.V. tolbutamide response. In 11 normal subjects on a standard diet containing liberal amounts of carbohydrate, the blood sugar response to tolbutamide was 1. Using one of his criteria may be noted that all but 1 r greater than 23 per cent of the initial value. In addition, the maximal fall in blood

ject previously tested while on a normal diet. It is of interest to note that

change in the curve can be seen. Similarly, performance of the test after ...ours of excessive carbohydrate intake and 2 hours after a high carbohydrate ...l produced no alteration in the response However, in 2 subjects in whom ...bohydrate was eliminated for a period of 3 to 7 days and who were main...ed on isocaloric diets, high in protein and fat, 2 different responses were ...ed (FIGURE 5) Subject H B showed a response which was entirely normal. ...bohydrate deprivation produced a change in response in subject M K, ..., incidentally, has a very extensive family history of diabetes Prior to ...bohydrate deprivation, the time of maximal blood sugar fall following ...utamide was 45 min with a fall at 30 min of 31 5 per cent Following ...rivation, the time of maximal fall was 60 min, and the fall at 30 minutes



...IGURE 6 Composite curves with the spread of the 30-min values indicated The bar ...cates the 23 per cent level of blood sugar fall

Effect of Steroids on I V Tolbutamide Response

...lood sugars In these experiments 26 subjects were maintained on stand... diets and then were pretreated with Decadron In some cases the same ...ject was retested with Prednisone in order to determine whether the two ...roids were comparably diabetogenic The effect of steroids on the tolbuta...de response may be seen in FIGURE 6 Two types of response were noted ...e curve, which was shown by 18 subjects, was termed normal since it most ...sely resembled the shape of the original or nonsteroid tolbutamide response ...e second type of response, termed abnormal, was shown by 8 subjects, it ...thfully reproduced the curve seen in diabetics. It is noteworthy that the ...ponse to steroids is manifested in all subjects by a delay in the time of ...ximal blood sugar depression In the normal curve the delay is from 30 ...n to 45 to 60 min, while in the abnormal curve, even at 90 min. the blood ...gar is still falling In addition to the time delay and change in shape of the ...ve, absolute changes in blood sugar level were noted following administra-

subjects showing a fall at 30 min. which was less than 23 per cent.

FIGURE 7 shows the absolute blood sugar levels and the ranges observed in all the subjects studied. Several points may be noted. First, there is a stepwise increase in the initial blood sugar level as seen by comparing the three curves. Second, the actual fall in blood sugar in the normal steroid curve is as great, or greater than that noted in the initial tolbutamide curve. This is not true in the case of the abnormal curve. Examination of FIGURE 8 shows the distribution of blood sugar values and indicates that a diabetic curve is to be expected following steroids if the initial blood sugar is above

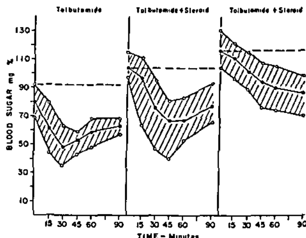


FIGURE 7 Mean and range of blood sugar values following tolbutamide with and without steroid pretreatment

115 0 mg per cent or if the 30- and 45-min. samples are above 96 0 and 86 0 mg per cent, respectively In view of the overlap of blood sugar levels in the two curves, the combined observations of the shape of the curve and the level of blood sugar are required to call a response abnormal. In 3 subjects tested with Prednisone and Decadron, both steroids produced comparable changes. It is important to note that all subjects who gave an abnormal test with steroids showed a normal test with tolbutamide alone.

Nonesterified fatty acids (NEFA) of blood FIGURE 9 shows the nonesterified fatty acid changes, subsequently referred to as NEFA, observed during the course of the blood sugar responses previously noted. Aside from the higher initial level of NEFA in both groups treated with steroids, there is a remarkable similarity in the shape and magnitude of response in all three curves The fact that the abnormal sugar curve was associated with a normal NEFA response seemed unusual in view of the demonstrated relationship to NEFA of blood sugar responses.

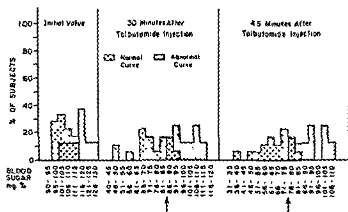


FIGURE 8 The position of the arrow indicates the level of blood sugar above which all curves were abnormal

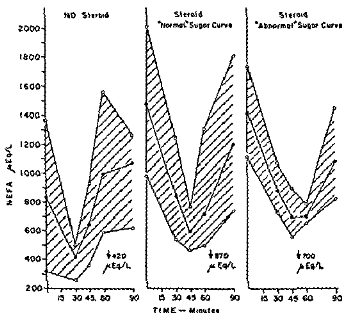


FIGURE 9 Mean values and range of NEFA plotted.

Relationship to family history of diabetes. The abnormal blood sugar curve in response to steroids was observed in 8 subjects. Of this group 6 gave a positive family history of diabetes.

Discussion

The immediate response to intravenous tolbutamide appears to depend upon the release of insulin from the pancreas. The data obtained from studies using dogs also suggest that a simultaneous decrease in hyperglycemic factor may occur and contribute to this effect. It is of interest to note that the response appears to be short-lived and declining by the 30 min. point. However, since no observations were made after 30 min., it is not possible to state whether insulin secretion is sustained or reactivated therafter.

On the basis of these data obtained from animal studies, one can attempt to
It seems likely that
ood sugar represents
The simultaneous
fall in NEFA noted is similar to the one produced by insulin and suggests a

an epinephrine effect Holzbauer and Vogt[9] have shown in the dog given insulin that epinephrine in peripheral blood is released almost as soon as the blood sugar starts to fall and continues to be released throughout the fall, gradually decreasing in quantity as the blood sugar rises It is interesting that their data show that there is a less pronounced, but definite rise in epinephrine with as small a blood sugar fall as 15 mg. per cent. An additional point of interest relates to the demonstration that epinephrine causes a rise[10] in NEFA These observations appear to explain the characteristics of the normal blood sugar and NEFA response to tolbutamide.

It is interesting that 24-hour starvation or excessive carbohydrate intake resulted in no changes in tolbutamide response. On the other hand, restriction of carbohydrate, known to induce a decrease in pancreatic insulin[11] among

When steroid pretreatment was given, the tolbutamide response was significantly modified Since the major effect of these steroids (Decadron, Prednisone) is to increase gluconeogenesis and raise the blood sugar level, it is not surprising that elevated blood sugars were observed in all patients. It seems likely that this rise in blood sugar occurring over an 8½-hour period prior to the tolbutamide injection is accompanied by an increased release of the stored insulin of the pancreas Injection of tolbutamide at this time presumably tests the pancreatic reserve and the individual with diminished reserve shows a significantly smaller fall in blood sugar. This was apparent in the 2 types of curves noted and one might use the term prediabetic for the curve suggesting decreased insulin stores.

However, does the decreased reserve of insulin adequately explain the different shape of the curve, namely the continual decline of blood sugar in the pre-

diabetic and diabetic? Since the NEFA curves were similar in the normal and prediabetic groups, showing a secondary rise, it seems probable that *epinephrine was liberated in both groups If the level of epinephrine required* to increase NEFA is less than that required to restore the blood sugar to normal levels, it could be assumed reasonably that more epinephrine was released in the normal than in the prediabetic or diabetic This could explain the failure to see a secondary rise in the abnormal sugar curves

On the other hand, progression from the initial tolbutamide curve to the steroid-normal curve and, ultimately, to the steroid-prediabetic curve (FIGURE 6), is manifested by a delay in the time of maximal blood sugar fall This delay could represent the conversion of preinsulin to insulin, when the stores [illegible]

These challenging questions are unanswered and revolve about pancreatic metabolism and the mechanism by which tolbutamide effects a release of pancreatic insulin

The data obtained relative to prediabetics is based on numbers too small, and follow-ups too short to be conclusive However, the family history of diabetes appears to increase the probability of an abnormal tolbutamide response following steroids

Summary

Studies have been presented that were designed to explain the nature of the normal, prediabetic, and diabetic blood sugar response to intravenous tolbutamide.

Acknowledgments

We thank C J O'Donovan for supplying the sample of sodium tolbutamide and N Capeci for the Prednisone and Decadron and Mary B Koch and Eudoxia Hatch for their technical assistance

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RELATIVE EFFECTIVENESS OF NEWER ORAL AGENTS IN THE REGULATION OF DIABETIC PATIENTS IMPERFECTLY CONTROLLED BY TOLBUTAMIDE STUDIED WITHIN THE FRAMEWORK OF A TENTATIVE SUBCLASSIFICATION OF THE DISEASE

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Specific correction of the underlying physiological derangement or deficit is

lected

In the present study, we have attempted to evaluate, within the framework of a tentative clinicophysiological subclassification of diabetes, the relative effectiveness in this disease of the presently available oral blood glucose-lowering agents, both singly and in combination.

Subclassification of Diabetes Mellitus

It is generally agreed that diabetes can be divided into at least 2 major groups (TABLE 1) The first group, designated as Type 1, is perhaps best de-

clinical type is characterized physiologically by the presence of insulin in the beta cells[2] and by insulinlike activity of endogenous origin[3, 4] in the plasma.

Type 2 diabetes, more commonly referred to as juvenile, growth-onset, unstable, or labile diabetes, is perhaps more aptly described as ketoacidosis

p

c

o

and might, therefore, be described as "abinsulinogenic."

No further consideration will be made in this report of this latter group nor of those transitional cases that are difficult to fit into either of these two categories

Subclassification of Type 1 Diabetes

In order to provide a basis for clinical comparison, Type 1 diabetics have been [illegible] ness of [illegible] The [illegible] a subclassification will be discussed below.

Patients in whom mean fasting normoglycemia is maintained with tolbu-

* This term, recently proposed by Wilder,[1] seems preferable to the more commonly used terms, stable, adult, or maturity-onset diabetes

tamide are designated as the tolbutamide-responsive subgroup, those in whom a blood-glucose response to maximal doses of tolbutamide takes place but falls short of fasting normoglycemia are designated as the tolbutamide-hyporesponsive subgroup. In this category blood sugar control ranges from near-normal to entirely unacceptable levels. Finally, those Type 1 diabetics in whom no significant blood sugar-lowering effect attributable to tolbutamide therapy can be discerned, are designated tolbutamide-unresponsive.

Selection of Patients

The tolbutamide-responsive subgroup, as defined above, is ideally controlled according to usual clinical criteria. Mean fasting normoglycemia has been achieved without any penalty in the form of serious hypoglycemia or drug toxicity. Furthermore, as will be pointed out later, it is possible to defend this therapy on physiological grounds. Since the need for additional methods of blood sugar control seems least in this tolbutamide-responsive subgroup, the patients selected for this study were drawn primarily from the ranks of the

TABLE 1
CLASSIFICATION OF DIABETES

Type 1 Ketoacidosis-resistant			Type 2 Keto-acidosis prone
Tolbutamide responsive (FBS normal on tolbutamide administration)	Tolbutamide hyporesponsive (partial response ranging from near normal FBS to unacceptably high levels)	Tolbutamide unresponsive (no detectable FBS response to tolbutamide)	

tolbutamide-hyporesponsive and tolbutamide-unresponsive subgroups (93 per cent).

The patients were members of the Parkland Memorial Hospital Metabolic Clinic or the Veterans Hospital Diabetes Study Clinic and consequently were observed at weekly intervals as outpatients.

Methods

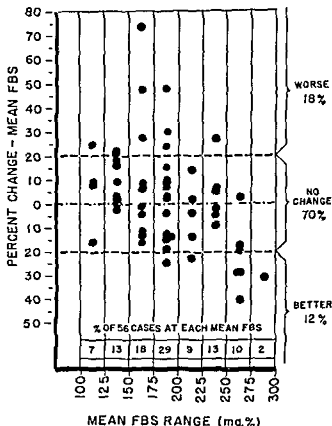
Weekly fasting blood glucose levels were determined in Parkland Hospital patients by a modification of the ferricyanide method of Hoffman,[5] using the *Technicon Corporation Autoanalyzer.**

Therapy with chlorpropamide, metahexamide, and Phenformin (DBI) was begun at minimal dosage levels and the dose was raised progressively each week until a response in fasting blood glucose level (FBS), the appearance of drug toxicity, or an unsatisfactory response to the drug was observed. Persistence of FBS levels at or above the tolbutamide baseline values despite 4 to 6 weeks of a drug in maximal doses was considered to warrant change to another agent. The order of agents used following discontinuation of tolbutamide therapy was

* Kindly performed by the laboratory of Morton F. Mason.

not randomized and was, in general, as follows: metahexamide, chlorpropamide, DBI, and tolbutamide plus DBI.

The mean FBS level of each patient while on tolbutamide therapy was compared with the mean FBS level while on the various newer therapeutic regi-



significant

mens. Percentage of change in mean FBS level following conversion from tolbutamide to a newer oral drug was then calculated

Results

Metahexamide. Fifty-six patients were changed from tolbutamide to metahexamide therapy. Metahexamide therapy was begun at dosage levels of 100 to 150 mg./day, administered in divided doses before breakfast and dinner The dose was raised each week by 50 mg until either a decline in FBS to normal

or near-normal levels was noted, drug toxicity appeared, or 4 to 6 weeks of therapy at a dosage level of 300 to 400 mg /day had proved to be ineffective.

In FIGURE 1, on the horizontal axis, the range of mean FBS level during prior tolbutamide therapy is indicated for each of these patients, the per cent change from these levels during metahexamide therapy is shown on the vertical axis A change in mean FBS level of less than 20 per cent was regarded as of doubtful clinical significance Thirty-nine patients (70 per cent of this series)

TABLE 2
MEAN AND RANGE OF FBS LEVELS IN PATIENTS IN WHOM CONVERSION FROM TOLBUTAMIDE TO METAHEXAMIDE WAS ASSOCIATED WITH A DECLINE IN MEAN FBS OF 20 PER CENT OR MORE

Patient	Tolbutamide	Metahexamide
1*	145 (110 to 180); 296 (220 to 335)	188 (175 to 213)
2	272 (238 to 282)	194 (101 to 280)
3	179 (160 to 190)	138 (123 to 152)
4	293 (263 to 203)	197 (194 to 264)
5	266 (284 to 222)	160 (153 to 189)
6	201 (150 to 253)	156 (129 to 186)
7	186 (152 to 249)	140 (114 to 166)

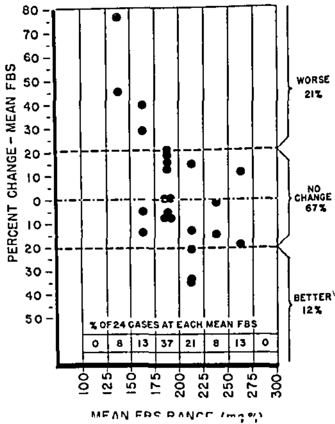
* This patient had had 2 separate courses of tolbutamide therapy, the first of which was satisfactory, the second unsatisfactory Conversion to metahexamide was preceded by an interval of insulin therapy

The blood sugar range of the 7 patients who showed a significant reduction in mean FBS level while on metahexamide is examined more closely in TABLE 2 In all but the first patient, in whom interpretation is difficult because of a long hiatus between tolbutamide and metahexamide courses, the improvement over

control following conversion to metahexamide therapy was most prevalent among patients in whom the mean FBS level while receiving tolbutamide had

Chlorpropamide Twenty-four patients were converted from tolbutamide to chlorpropamide The drug was administered in a single daily dose beginning

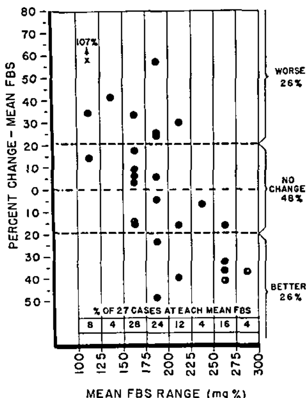
at 500 mg /day. The dose was increased by 125 to 250 mg /wk. until a decline in FBS to normal or near-normal levels, the appearance of drug toxicity, or 4 to 6 weeks of unsatisfactory FBS response at dosage levels of up to 1 0 gm /day had been noted FIGURE 2 indicates the per cent change in mean FBS from tolbutamide baseline levels noted during therapy with chlorpro-



pamide. Improvement was noted in 3 patients (12.5 per cent), deterioration in 5 patients (20 per cent), and in 16 patients (67 5 per cent) no significant change was observed. The range of fasting blood glucose levels of the 3 cases in whom improvement was noted is shown in TABLE 3. The improvement in these 3 cases, 2 of whom had been inadequately controlled by metahexamide as well as by tolbutamide, was striking, and in each case the FBS level was maintained within a clinically acceptable range

Phenformin. Twenty-seven patients were converted from tolbutamide to

tained or until a "bad taste" or anorexia appeared Failure to reduce the



cal axis.

FBS level after 3 weeks of therapy at maximal subtoxic levels was considered evidence of drug failure

Seven patients (26 per cent) showed significant improvement in mean FBS control after conversion from tolbutamide to DBI, 8 patients (30 per cent) appeared to deteriorate in this respect, while 12 subjects (44 per cent) were considered to be unchanged (FIGURE 3) The magnitude of improvement exceeded 30 per cent in 6 of the 7 improved cases

* Product of United States Vitamin and Pharmaceutical Corporation, New York, N Y.

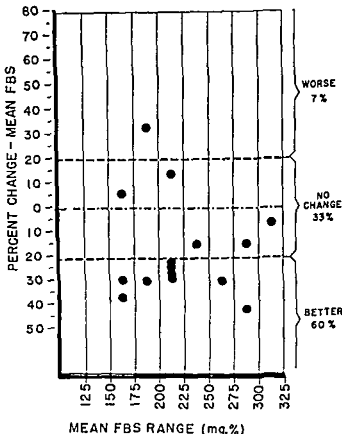


TABLE 3
MEAN AND RANGE OF FBS LEVELS IN PATIENTS IN WHOM CONVERSION FROM TOLBUTAMIDE TO CHLORPROPAMIDE WAS ASSOCIATED WITH DECLINE IN MEAN FBS OF 20 PER CENT OR MORE

Patient	Tolbutamide	Metahexamide	Chlorpropamide
1	207 (193 to 248)	214 (135 to 296)	163 (129 to 175)
2	202 (201 to 206)		132 (100 to 172)
3	218 (207 to 289)	250 (195 to 286)	144 (96 to 200)

TABLE 4 shows the ...

... would appear ...

Combinatio ...
with tolbutar ... hom had been ideal
... The order ...

... OR MORE

Patient	Tolbutamide	Metahexamide	Chlorpropamide	Phenformin
1	255 (201 to 300)	263 (201 to 291)	285 (251 to 300)	173 (144 to 192)
2	203 (125 to 250)	177 (131 to 201)	178 (80 to 268)	152 (124 to 178)
3	272 (261 to 282)	194 (101 to 260)	—	119 (101 to 157)
4	196 (135 to 280)	256 (150 to 350)	195 (136 to 265)	110 (102 to 127)
5	189 (120 to 273)	236 (90 to 470)	—	146* (127 to 188)
6	262 (110 to 335)	188 (175 to 213)	—	169 (154 to 183)
7	294	—	—	189 (189 to 200)

* Since the preparation of this table this patient has become unresponsive to Phenformin

... min was added directly to an un-
... utamide was considered the basic
... supplementary agent to be given in
... or optimal FBS control The
... ly
... duration revealed substantial
... mean FBS level over that of prior tolbutamide therapy in 60 per cent of these patients, no change in 33 per cent, and deterioration in only 7 per cent (FIGURE 4) The means and ranges of FBS levels in the improved cases are shown in TABLE 5

Toxicity The toxicity data are summarized in TABLE 6 and require little amplification *

The side effects of DBI, which are confined to the gastrointestinal tract, are not included in TABLE 6 since these symptoms will invariably appear when the limits of individual tolerance are exceeded. These vary widely from patient to patient (75 to 400 mg in our experience). In this respect the drug is similar to digitalis

It should be stressed that in TABLE 6 the term hypoglycemic symptoms refers to symptoms compatible with hypoglycemia that were relieved by food and does not imply laboratory documentation. Although the danger of hypo-

TABLE 5

MEAN AND RANGE OF FBS LEVELS IN PATIENTS IN WHOM CONVERSION FROM TOLBUTAMIDE TO TOLBUTAMIDE PHENFORMIN COMBINATION THERAPY WAS ASSOCIATED WITH A DECLINE IN MEAN FBS OF 20 PER CENT OR MORE

Patient	Tolbutamide	Metahexamide	Chlorpropamide	Phenformin	Tolbutamide-Phenformin
12*	294	—	—	189 (184 to 200)	125 (97 to 156)
13*	185 (136 to 210)	193 (163 to 245)	170 (135 to 180)	220 (144 to 250)	133
11*	293 (263 to 308)	197 (144 to 270)	—	249 (242 to 256)	130
4	255 (201 to 300)	263 (255 to 291)	284 (251 to 300)	172 (144 to 192)	119 (80 to 139)
5	172 (95 to 250)	—	—	—	126 (116 to 135)
6	234 (146 to 421)	300 (164 to 396)	—	259 (190 to 348)	149 (140 to 158)
7	188 (137 to 238)	181 (124 to 225)	189 (124 to 220)	181 (103 to 240)	134 (129 to 140)
8	215 (177 to 300)	—	—	—	156 (140 to 168)
9	206 (199 to 215)	171 (111 to 202)	250 (224 to 275)	—	155 (115 to 186)
3	202 (181 to 222)	—	132 (100 to 172)	—	150 (117 to 177)
1	270 (269 to 285)	219 (151 to 300)	220 (195 to 217)	277 (216 to 280)	167 (140 to 188)
2	191 (148 to 261)	—	—	—	157 (144 to 178)
10	272 (267 to 278)	—	—	—	136 (114 to 158)

* Not included in FIGURE 4 because of inadequate baseline observation periods

glycemia during therapy with tolbutamide alone is slight, and with Phenformin *alone* probably absent, its incidence when these 2 agents are used in combination is as yet uncertain

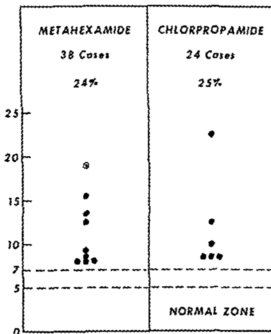
During both metahexamide and chlorpropamide therapy, serial determinations of alkaline phosphatase levels revealed in 24 and 25 per cent, respectively, of patients followed, a level of 7 Bodansky units (B U) or above on at least 1 occasion The peak value occurring in each such patient is recorded in FIGURE 5 In many of these cases the elevation in alkaline phosphatase level was transient, returning to normal despite continued therapy, often to recur In some, however, a progressive rise, at times to alarming levels, was observed As a check on the technical accuracy of the clinical laboratory a blood specimen was

TABLE 6

INCIDENCE OF SIDE EFFECTS DURING THERAPY WITH THE LONG-ACTING SULFONYLUREAS

	Metahexamide (82 patients)	Chlorpropamide ([illegible] patients)
Hypoglycemic symptoms	4 (5)%	2 (5%)
Nausea	7 (9%)	2 (5%)
Vomiting	2 (2.5%)	0
Abdominal pain	2 (2.5%)	1 (2.5%)
Diarrhea	0	2 (5%)
Weakness	0	2 (5%)
Rash	1 (1.3%)	2 (5%)
Drug fever	1 ?(1.3%)	0
Jaundice	1 (1.3%)	0
Elevation in alkaline phosphatase > 7 B U	(24%)*	(25%)*

* See FIGURE 5



The side effects of DBI, which are confined to the gastrointestinal tract, are not included in TABLE 6 since these symptoms will invariably appear when the limits of individual tolerance are exceeded. These vary widely from patient to patient (75 to 400 mg in our experience) In this respect the drug is similar to digitalis

It should be stressed that in TABLE 6 the term hypoglycemic symptoms refers to symptoms compatible with hypoglycemia that were relieved by food and does not imply laboratory documentation Although the danger of hypo-

TABLE 5

MEAN AND RANGE OF FBS LEVELS IN PATIENTS IN WHOM CONVERSION FROM TOLBUTAMIDE TO TOLBUTAMIDE-PHENFORMIN COMBINATION THERAPY WAS ASSOCIATED WITH A DECLINE IN MEAN FBS OF 20 PER CENT OR MORE

Patient	Tolbutamide	Metahexamide	Chlorpropamide	Phenformin	Tolbutamide-Phenformin
12*	294	—	—	189 (184 to 200)	125 (97 to 156)
13*	185 (136 to 210)	193 (163 to 245)	170 (135 to 180)	220 (144 to 250)	133
11*	293 (263 to 308)	197 (144 to 270)	—	249 (242 to 256)	130
4	255 (201 to 300)	263 (255 to 291)	284 (251 to 300)	172 (144 to 192)	119 (80 to 139)
5	172 (95 to 250)	—	—	—	126 (116 to 135)
6	234 (146 to 421)	300 (164 to 396)	—	259 (190 to 348)	149 (140 to 158)
7	188 (137 to 238)	181 (124 to 225)	189 (124 to 220)	181 (103 to 240)	134 (129 to 140)
8	215 (177 to 300)	—	—	—	156 (140 to 168)
9	206 (199 to 215)	171 (111 to 202)	250 (224 to 275)	—	155 (115 to 186)
3	202 (181 to 222)	—	132 (100 to 172)	—	150 (117 to 177)
1	270 (269 to 285)	219 (151 to 300)	220 (195 to 217)	277 (216 to 280)	167 (140 to 188)
2	191 (148 to 261)	—	—	—	157 (144 to 178)
10	272 (267 to 278)	—	—	—	136 (114 to 158)

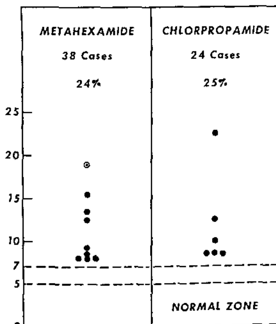
* Not included in FIGURE 4 because of inadequate baseline observation periods

occasion. The peak value occurring in each such patient is recorded in FIGURE 5. In many of these cases the elevation in alkaline phosphatase level was transient, returning to normal despite continued therapy, often to recur. In some, however, a progressive rise, at times to alarming levels, was observed. As a check on the technical accuracy of the clinical laboratory a blood specimen was

TABLE 6
INCIDENCE OF SIDE EFFECTS DURING THERAPY WITH THE LONG ACTING SULFONYLUREAS

	Metahexamide (82 patients)	Chlorpropamide (37 patients)
Hypoglycemic symptoms	4 (5)%	2 (5%)
Nausea	7 (9%)	2 (5%)
Vomiting	2 (2 5%)	0
Abdominal pain	2 (2 5%)	1 (2 5%)
Diarrhea	0	2 (5%)
Weakness	0	2 (5%)
Rash	1 (1 3%)	2 (5%)
Drug fever	1 ?(1 3%)	0
Jaundice	1 (1 3%)	0
Elevation in alkaline phosphatase > 7 B U	(24%)*	(25%)*

* See FIGURE 5



obtained from each of 40 patients receiving Phenformin and tolbutamide. The highest alkaline phosphatase level in this group was 4 8 B.U.

In 1 patient, a 52-year-old Negro female receiving metahexamide in doses of 100 to 350 mg over a 10-week period, evidence of clinical liver disease was



nuclears. Courtesy -

noted. This patient was hospitalized because of anorexia, malaise, abdominal pain, and jaundice. Serial liver biopsies, kindly studied by Charles T. Ashworth, were considered to show postnecrotic cirrhosis of probable recent onset (FIGURE 6), attributable either to viral hepatitis or to a drug reaction This patient's recovery has been uneventful

Discussion

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possibility of spontaneous alteration in diet, of unrecognized infection, and of psychic factors, is most difficult to evaluate in outpatients and must be considered in the interpretation of these results Furthermore, the anorexigenic

of conclusions

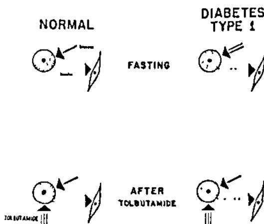
Substantial improvement in control of mean FBS level occurred in only 12 per cent of patients imperfectly regulated by tolbutamide following conversion to metahexamide or chlorpropamide therapy, an unimpressive salvage rate The possibility of acute or chronic cholangiolar and hepatic injury by these agents seems to preclude, at least for the present, their use for routine control of blood sugar, save in the exceptional case in which no other regimen, oral or parenteral, is effective

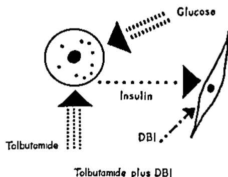
DBI alone appeared to have a higher salvage rate of (26 per cent—7 patients) among tolbutamide-hyporesponsive patients The magnitude of the improve-

weeks on combination therapy Because of the small size of this series and the short period of observation any final conclusion in respect to this group must be deferred The initial results, however, are most encouraging (FIGURE 4).

most reasonable The functional lesion in tolbutamide-responsive diabetics may well consist of a hyporesponsiveness in terms of insulin release per unit of time to a given level of betacytotropism Thus, in order for such a beta cell to

maintain a normal insulin output, an elevated blood sugar level is required (FIGURE 7)* Tolbutamide, in some way, may diminish this reluctance of the beta cell to release its insulin, thereby permitting a normal insulin output at a





Tolbutamide plus DBI

blood glucose level

* Since preparation of this manuscript, H S Seltzer and W. Smith have reported evidence to support this hypothesis.[4]

both clinical and, tentatively, on physiological grounds and may well be the treatment of choice for this category of diabetes

The difference between the tolbutamide-hyporesponsive subgroup and the tolbutamide-responsive group may be purely quantitative, or additional defects may be present In any case a more potent sulfonylurea or betacytotropin of established safety and superior effectiveness would seem appropriate Until such an agent is available, the use of tolbutamide as the basic therapy with [illegible]

[illegible] this group exogenous insulin must, of course, continue to be the mainstay of therapy, although supplementation with one or more of the oral agents may be helpful

Summary and Conclusions

Only 12 per cent of patients imperfectly controlled with tolbutamide undergo substantial improvement in mean FBS levels when converted to metahexamide or to chlorpropamide Increase in the level of alkaline phosphatase was noted in 25 per cent of the patients receiving these agents Postnecrotic cirrhosis developed in 1 metahexamide-treated patient Until the risk of cholangiolar and hepatic damage by these agents has been finally clarified, their routine use would seem to be precluded

[illegible] per cent of a small series

An attempt has been made to develop a clinicophysiological subclassification of diabetes based on responsiveness to tolbutamide This may facilitate clini- [illegible]

Acknowledgment

We express our gratitude to Charles T Ashworth, Chairman of the Department of Pathology, University of Texas Southwestern Medical School for the pathological examination reported herein, and to Morton F Mason for the laboratory work on the Parkland Hospital Patients

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COMBINED INSULIN-TOLBUTAMIDE THERAPY IN THE MANAGEMENT OF INSULIN-DEPENDENT DIABETES

Maximilian Fabrykant and Benjamin I Ashe
Department of Medicine, New York University, Post-Graduate Medical School, New York, N Y

Early observations on the sulfonylurea compounds made by German clinicians indicated that these substances may be successfully used in the management of mild diabetes of the maturity-onset type, but are of no benefit in more severe forms of the disease and in patients who acquired it at an early age Since the compounds were found to be ineffective in depancreatized and alloxan-diabetic animals it was concluded that for their antidiabetic activity they need an adequate islet reserve and that, therefore, their use is contraindicated in the growth-onset type of diabetes and in patients who require large doses of insulin At the same time, however, it was also noted that about one third of patients with the maturity-onset diabetes who were considered to be good candidates for the use of sulfonylureas failed to respond to these compounds The question of why these patients were refractory to sulfonylurea preparations remained unanswered

From unpublished experimental work with 2254 R P , the compound used by the French investigators in their original studies of hypoglycemic thiodiazols, we have gained the impression that the presence of an intact pancreas does not insure a hypoglycemic response to the employed thiodiazol and that some extrapancreatic factors may be involved in the mechanism by which this thiodiazol exerts its influence on the blood sugar content With these thoughts in mind we assumed that failure of diabetic patients to respond to the newer sulfonylureas may be related to extrapancreatic factors and have decided to use these compounds in conjunction with insulin in patients whose diabetes cannot be controlled without the employment of this hormone Because these studies were begun shortly after the compounds became available in the United States,[1,2] we are able to present observations that extend over a period of more than three years and include a large series of patients in whom the use of these compounds is generally thought to be contraindicated.

Clinical Material and Results

TABLE 1 presents the clinical data on our group of 131 patients with insulin-

with the most ingenious and meticulous schemes of insulin administration FIGURE 1 illustrates our tribulations during early phases of such therapy, and in particular the failure of Orinase to prevent rapid deterioration of metabolic control in these patients when their insulin dosage is reduced by as little as 2 or 4 U daily

Since diabetes is a chronic disease, long-term effects of an antidiabetic medication seemed to us more important than the immediate effects noted in acute

experiments Furthermore, we thought it more profitable to study an antidiabetic medication not only as a substitute for insulin, but on its own merits. For these reasons we have continued the use of Orinase in our labile diabetics in spite of the discouraging outcome of short-term observations In subsequent patients the administration of the drug was initiated without a change in insulin dosage, but even with this regimen it took from 2 to 8 weeks before an improvement from the combined therapy became clearly noticeable. A favorable response was obtained in 35 patients who, thus far, have been continued on the combined therapy for periods of 12 to 40 months. All 35 experienced stabilization of their diabetic control together with a feeling of general stability and well-being In 21 of them there was also a substantial saving in insulin dosage that is reflected in gradual reduction by 27 to 50 per cent of the pretreatment

TABLE 1
CLINICAL DATA ON 131 INSULIN-DEPENDENT DIABETICS GIVEN COMBINED INSULIN-ORINASE THERAPY (C.I.-O.T.)

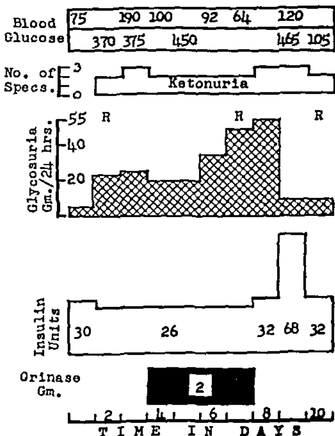
No. of cases	Clinical type	Diabetes		Insulin prior to Orinase (units per day)	Response to C.I.-O.T.	
		Age at onset (years)	Duration (years)		Good	Poor
46	labile	6 to 40	4 mo. to 34 yr	28 to 132	35* (76%)	11‡ (24%)
27	stable mild	39 to 75	8 mo. to 24 yr	10 to 40	22** (81.5%)	5 (18.5%)
58	stable severe	11½ to 56	5 to 29	52 to 156	51† (88%)	7§ (12%)

dose. The remaining 11 patients with labile diabetes were not improved by adjunctive administration of Orinase. In 9 of them the drug was discontinued

three widely separated trial periods of 14 to 18 days, the use of the drug was abandoned after the short-term experiments.

In the group of stable diabetics given combined insulin-Orinase therapy, there were 27 with mild diabetes who required from 10 to 40 U. of insulin daily. T[illegible]
but after
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dosage, and in consequence were not taken off insulin entirely at any time Five of these mild diabetics derived no benefit from the combined therapy and, in consequence, the administration of Orinase in these patients was discontinued



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In the group of stable diabetics given combined insulin-Orinase therapy, there were 27 with mild diabetes who required from 10 to 40 U of insulin daily. Ten of these patients were initially well controlled on Orinase alone, but after 2 to 4 months broke out of control and were compelled to add small

Beneficial results were obtained in 76 per cent of 46 patients with labile and 86 per cent of 85 patients with stable diabetes Outstanding among such

patients, but the described general beneficial effects were not related to changes in insulin dosage

Observations herein reported and the fact that one third of patients regarded as good candidates for therapy with sulfonylureas are refractory to these compounds are taken to indicate (1) that responsiveness to sulfonylureas does not seem to depend exclusively on the pancreatic islets and (2) that extrapancreatic factors are operative in bringing about the clinical improvement in patients treated by the combination of insulin and Orinase

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Among the 58 patients with severe diabetes, 7 with onset of the disease between 7 and 11 years of age showed minimal or no response to Orinase. Even after 8 to 12 weeks on Orinase therapy, they still reacted with disruption of metabolism when their usual insulin dosage was decreased but slightly. By contrast, patients with a high insulin requirement who acquired the disease after

stability and well-being.

Discussion

In our series of 131 insulin-dependent diabetics beneficial effects from long-term combined insulin-Orinase therapy were obtained in 35 patients (76 per cent) with labile and 74 patients (86 per cent) with stable diabetes. The benefits consisted of (1) a sense of general stability and an enhanced feeling of well-being, (2) stabilization of diabetes in labile patients and ease and greater uniformity in diabetic control in both groups; (3) a decrease in insulin requirement of up to 50 per cent of the pretreatment dose in the labile and of up to 70 per cent in the stable group; and (4) a decrease in frequency and severity of hypoglycemic reactions in all patients including those whose insulin dosage remained unchanged

It is important to point out that greater uniformity in diabetic control and the general feeling of stability and well-being were not related to changes in insulin dosage. Such benefits were seen in patients who achieved a marked reduction in their insulin requirement as well as in those with little or no decrease in their pretreatment insulin dosage These facts indicate that the beneficial effects from adjunctive Orinase therapy are not confined to the hypoglycemic action of this substance and that the use of Orinase in combination with insulin provides benefits that cannot be attained with insulin alone

By what mechanism Orinase achieves the stabilizing and mood-raising effects is at present unknown It is, of course, tempting to postulate that the compound stimulates the production or release of endogenous insulin and in this way makes it possible to reduce or even to eliminate the administration of exogenous insulin and in so doing to obtain a smoother metabolic control. However we must keep in mind the fact that the feeling of stability and well-being associated with the use of Orinase is also seen in patients who have never been treated with insulin, and therefore it cannot be ascribed to cessation of insulin therapy in these patients As mentioned earlier, such general benefits are also seen in patients on combined insulin-Orinase therapy whose insulin requirement was not lowered by taking this sulfonylurea, that is, in patients who show no evidence of stimulation of islets by the compound We interpret these observations as indicative of extrapancreatic effects of sulfonylureas, particularly in relation to carbohydrate transformations in extrapancreatic body tissues

Summary and Conclusions

Long-term effects of Orinase as an adjunct to insulin therapy were studied in a group of 131 insulin-dependent diabetics for periods of 12 to 40 months.


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PHYSIOLOGICAL BASIS OF THE EFFECTIVENESS OF COMBINED INSULIN-TOLBUTAMIDE THERAPY IN STABLE DIABETES*

Sydney S. Lazarus and Bruno W. Volk

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Considerable physiological evidence has accumulated suggesting that the pancreatic beta cells are the main target for the hypoglycemic action of the sulfonylurea drugs. Loubatières originally demonstrated that these compounds lower the blood sugar in the normal and in the partially pancreatectomized dog but not in the completely depancreatized nor in the severely alloxan-diabetic animal.[1] Increased insulin output from the beta cells under the influence of these drugs is suggested also by experiments in which systemically ineffective dosages perfused directly through the pancreatic artery caused significant peripheral hypoglycemia.[2] Furthermore, in cross-circulation experiments hypoglycemia was noted in recipient animals after perfusion of the pancreatic arteries of donor dogs with the sulfa drugs.[3] Moreover, an increased insulin content of the pancreatic vein in the dog has been reported following acute administration of the sulfonylureas.[4] In more recent experiments it was shown electrophoretically that there is an increase of insulin in the pancreatic vein blood of calves after the administration[5] of D 860.

There is also, however, evidence that there may be extrapancreatic actions of these drugs which affect the blood sugar level. Thus chronic administration of tolbutamide is reported by some to reduce the degree of diabetes of completely depancreatized dogs maintained on small dosages of insulin.[6] Others could not demonstrate a significant action of these compounds on the rate of blood glucose removal, while the rate of conversion of lactose to glucose was diminished.[7] Furthermore, the rate of disappearance of intravenously infused pentose was shown to be increased threefold after insulin, but was not affected by the sulfonylureas.[8] Several workers have found a decrease of pyruvate and lactate after carbutamide, compared with an increase after insulin.[9,10] Still other investigators utilizing C^{14}-tagged glucose observed that after insulin administration in the rat there is a rapid fall in specific activity in the blood while, after tolbutamide, the fall in specific activity is no greater than in control anir
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pancreatic mechanisms that have been suggested to account for these findings include, particularly, enhancement of insulin action by inhibition of insulinase[13] and inhibition of hepatic glycogenolytic systems,[14,15] as well as some action on the central nervous system.[12]

Morphologically it has been shown, unequivocally, that when administered over prolonged periods the sulfonylureas produce degranulation of the pancreatic beta cells of rats, rabbits, and dogs (FIGURE 1).[16-19] The functional significance of beta-cell degranulation might be twofold. Theoretically the

* The work reported in this paper was supported in part by a grant from The Upjohn Company, Kalamazoo, Mich.


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administration of exogenous insulin caused beta-cell degranulation and reduced pancreatic insulin content.[20-23] In these instances it is assumed that the beta-cell degranulation is indicative of suppression of insulin formation. On the other hand, hyperglycemia due to glucose administration in the rat also causes beta-cell degranulation and diminished pancreatic insulin content.[24-26] Under the latter circumstances it is believed that although insulin synthesis is increased, the rate of liberation of insulin from the pancreas is so high that the amount of stored insulin, as represented by the granules, is reduced.

The degranulation of beta cells after sulfonylureas has been interpreted by some as indicative of suppression of insulinogenesis similar to that observed during chronic hypoglycemia induced by insulin administration.[14] The main argument advanced in favor of this theory was that beta-cell degranulation is not observed after very short-term administration of these drugs. However, in both the cortisone-treated rabbit[27] and the growth hormone-treated dog,[28] degranulation of beta cells which in both instances is due to increased insulin output also is not observed within the first 48 hours. An additional argument advanced to support the view that tolbutamide degranulation is due to depressed insulinogenesis is that it causes no histochemically demonstrable depletion of pancreatic islet zinc content.[29] However, this conclusion is not warranted since little is known as to the actual relationship between insulin, zinc, beta-cell granules, and alpha cells. Furthermore, this finding could be interpreted as disproof either of the suppression or the stimulation hypothesis.

Relation Between Tolbutamide-Induced Beta-Cell Degranulation and Hypoglycemia

In order to determine whether tolbutamide-induced degranulation of pancreatic beta cells was a primary effect of the drug or whether it was secondary to the
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was compared. It was found that although tolbutamide produced varying degrees and frequently complete degranulation of the pancreas, insulin produced no changes in the beta cells in the rabbit. In the second type of experiment, rat
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values below the normal fasting level (FIGURE 3). The animals which received glucose and the drug simultaneously showed, in most instances, complete loss of beta-cell granules (FIGURE 4). The rabbits that received glucose alone showed no beta-cell changes (FIGURE 5).

The results of these two experiments indicate that the hypoglycemia is not

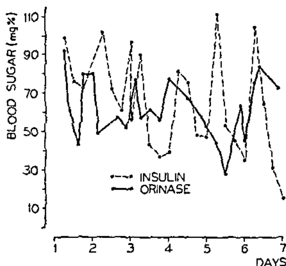
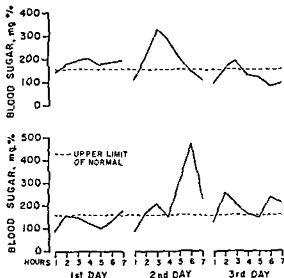


FIGURE 2 Representative blood sugar curves of 2 rabbits, 1 treated with 0.5 mg./kg. of tolbutamide intravenously twice daily, the other with 3 U. of NPH insulin subcutaneously once daily. Reproduced by permission from *Diabetes*.



the cause of the beta-cell degranulation and, furthermore, that the drugs produce degranulation even though hypoglycemia is prevented by simultaneous glucose administration. These findings are interpreted to mean that the sulfonylureas have a primary action on beta cells that increases the insulin out-



FIGURE 4 Islet of pancreas of rabbit infused with glucose alone showing normal beta-cell granulation (B) Alpha cells (*arrows*) are at the periphery. Aldehyde-fuchsin-trichrome stain × 380.

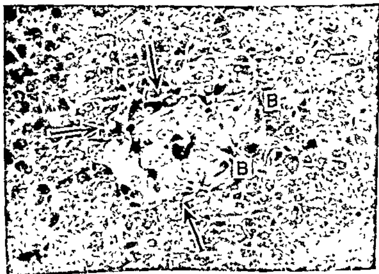


FIGURE 5. Islet of pancreas of rabbit infused with glucose and metahexamide showing degranulation of beta cells (B) Alpha cells (*arrows*) are dark. Aldehyde-fuchsin-trichrome stain ×380.

put and, thereby, causes degranulation This increased insulin output then would account for the drug-induced hypoglycemia

Relation Between Hypoglycemic Effectiveness of Tolbutamide and Pancreatic Insulinogenic Reserve

It was hypothesized that since the beta cell is the primary target of the sulfonylureas if these cells are functioning at maximum capacity, administration of the drug should cause no further increase in insulin output [12] This [illegible] duced by reducing the pancreatic mass and by administration of cortisone or growth hormone It has been shown that in certain phases of these hyperglycemic states the functional reserve of the pancreas is exhausted [27, 31, 32] For this purpose rabbits were treated with large dosages of cortisone and 80 per [illegible] beta cells were completely degranulated, administration of a single dose of the drug did not cause hypoglycemia (FIGURE 6) Furthermore, in metahypophyseal diabetic dogs, in which the pancreatic beta cells had been destroyed, daily administration of tolbutamide caused no improvement of the diabetic state [12] These experiments support the view that the hypoglycemic action of the sulfonylureas is mediated by stimulation of beta cells to increased insulin output and that with loss of pancreatic functional reserve the drugs become ineffective

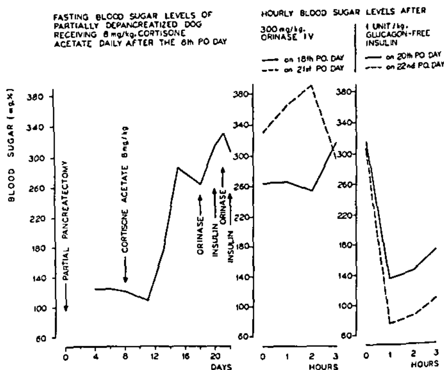
Clinical Considerations

The relative effectiveness of sulfonylureas in various types of human diabetes supports the idea that the pancreas is the primary target of these drugs Human diabetes mellitus is thought of as sometimes being due to lack of insulin and also frequently as being due to extrapancreatic factors that increase the demand for insulin or interfere with its action [33-35] Juvenile diabetes, which is frequently of the insulin-deficiency type usually does not respond to the sulfonylureas, and often the pancreas shows loss of islet tissue (FIGURE 7) Patients with maturity-onset diabetes may have considerable insulinogenic reserve with adequate, or only moderately diminished islet tissue (FIGURES 8 and 9) and do respond to the drug [36, 37] Furthermore, it has been shown that [illegible]

hyperglycemia increases the insulin output.

With this hypothesis in mind, a study was conducted on the effect of tolbutamide alone or administered together with insulin in a group of patients

with maturity-onset diabetes Those selected usually had fasting blood sugars above 200 mg per cent, despite the administration of at least 30 U. of insulin It was found, in general, that they fell into two categories. In one group, after gradual reduction and eventual omission of insulin, the blood sugar remained within the normal range on 1 gm. of tolbutamide daily alone (FIGURE 10) In the other type of patient, normoglycemic levels were maintained on 1 gm of tolbutamide together with approximately one half of the original dose



of insulin (FIGURE 11) In the latter group the blood sugar rose to much higher values when insulin was omitted completely

These studies demonstrate that tolbutamide administered together with insulin frequently has a synergistic effect in diabetic patients This is in keeping with the reports of others who noticed beneficial effects of the supplemental use of these drugs in stable, as well as in labile diabetics with high insulin requirement.[39-46]

The clinical data as well as the experimental findings allow this synergism to be explained on the basis that exogenous insulin administered alone depresses its endogenous secretion, whereas when tolbutamide is administered together



FIGURE 7 Pancreas of patient with juvenile diabetes There is paucity of insular tissue (The pancreatic tissue was received through the courtesy of P M Le Compte) Aldehyde fuchsin stain ×95

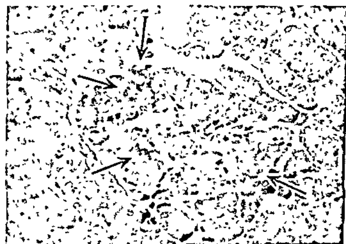


FIGURE 8 Islet of pancreas of patient with maturity-onset diabetes which is not readily distinguishable from the normal showing beta-cell granulation (*arrows*) Aldehyde-fuchsin stain ×250

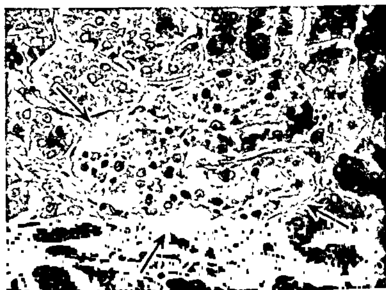


FIGURE 9 Pancreatic islet of patient with maturity-onset diabetes, showing partial hyalinization (*arrows*) Periodic-acid-Schiff-trichrome stain ×380.

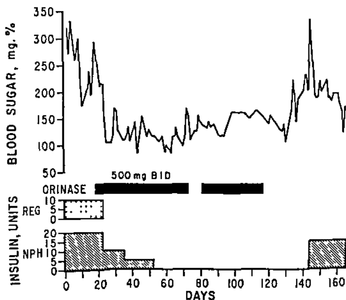


FIGURE 10 Blood sugar curve of diabetic showing better control with 1 gm of tolbutamide daily alone than with a mixture of 20 U of NPH and 10 units of regular insulin

with insulin, endogenous production of the hormone is maintained. This apparently allows for smoother and better metabolic control of the diabetes than when insulin is given alone. The findings imply that the sulfonylureas apparently act as a more effective insulinogenic stimulus than does hyperglycemia and that in some instances diabetes may result from a loss of responsivity of the pancreas to increase in the blood sugar level. The gradual loss of effectiveness of the sulfonylureas reported in some diabetics[16] may be explained as due

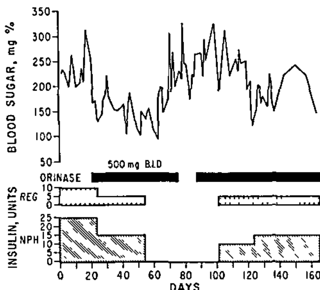


FIGURE 11. Blood sugar curve of diabetic showing better control with combined tolbutamide-insulin treatment than with either alone.

to maximal utilization in these individuals of the insulinogenic reserve which is, however, inadequate to maintain metabolic normality.

Summary

It has been demonstrated that the sulfonylureas cause degranulation of pancreatic beta cells. Furthermore, it has been shown that this pancreatic beta-cell degranulation is not due to the hypoglycemia which accompanies administration of the drug but is rather a primary effect of the drug on the beta cells. This degranulation is, therefore, considered to reflect increased insulin output from the beta cells under the influence of the drug. It was demonstrated furthermore that when the pancreas is functioning at maximal capacity and there is no insulinogenic reserve, as indicated by complete degranulation of the beta cells, the sulfonylureas do not cause hypoglycemia.

This finding is interpreted to mean that in order for the sulfonylureas to be effective, some pancreatic insulinogenic reserve must be present. With these ideas in mind a study was undertaken of the effectiveness of tolbutamide either alone or in combination with insulin in a group of selected diabetic patients with maturity-onset diabetes who were poorly controlled despite the fact that they received at least 30 U. of insulin daily. It was found that frequently good control could be obtained with a reduced amount of insulin together with a standard dose of 1 gm of tolbutamide daily. On the other hand, occasional patients responded to the drug alone. The tolbutamide-insulin synergism in these patients is considered as signifying that tolbutamide stimulates endogenous pancreatic function despite the depressing effect of concomitant exogenous insulin These findings suggest that most maturity-onset diabetics have varying amounts of residual pancreatic insulinogenic ability which is not utilized when they are treated with insulin alone. Furthermore, it is suggested that diabetic patients who respond to tolbutamide alone probably have adequate insulinogenic reserve but a defect in the normal mechanism for controlling the blood sugar by increasing the insulin output.

Acknowledgment

The microphotographs were prepared by Herbert A. Fischler.

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46

THE BIGUANIDES· THEIR ROLE IN THIS ERA OF THE PRECISE TOOL

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Joslin Clinic and New England Deaconess Hospital, Boston, Mass

This "brave new world" of oral hypoglycemic agents is not of contemporary or even recent origin (FIGURE 1) In 1918 Watanabe[1] reported a correlation between the effect of guanidine on intermediary metabolism and decreased blood glucose levels The intervening four decades have seen the rise and fall of many compounds At present, there is interest in a freshet of oral agents whose common denominator is the ability to assist in lowering blood sugar levels. The sulfonylureas (carbutamide, tolbutamide, chlorpropamide, and metahexamide) have been and are being explored and delineated The ex-
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nylurea compounds undeniably lowered blood sugar levels in many patients, their scope of action was clearly limited and offered no succor to the severe and brittle diabetic, our study series with biguanides started in December 1956

The Biguanides

The biguanides u
(DBV) biguanide
of hypoglycemic act
450 mg /day in divided doses, with an average effective dose of 150 mg /day Certain patients also required a supplementary dose of insulin The area between the effective dose and the level at which side effects occur proved to be a relatively narrow one in the more severe cases with unstable diabetes

Material

In the past 28 months (December 1956 to April 1959) more than 350 patients

to have diabetes 2 years or longer Duration of diabetes was 1 to 37 years, while the average known duration was 9 4 years

(3) Age of the 244 cases studied was 2 to 80 years (average 37 5 years, *see* TABLE 2).

(4) Thirty-two patients had never taken insulin previously The other 212 averaged 32 units daily with a range of 4 to 136 units

Methods

The routine of study and criteria used have been previously published,[4] and an extensive use was made of placebos to demonstrate the activity of diabetes.

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The criteria used were adequate but not perfect, since many factors may contribute to lowering of blood sugar levels, and diabetes is a disease of occasional remissions The report of Balodimos[5] of 100 diabetic patients is a reminder of the long-known fact that hospitalization and strict diet can lower insulin dosage by more than 50 per cent Reinforcement with placebo studies has made the criteria more acceptable

Results

According to the classification in TABLE 3, the results are as shown in TABLE 4 Combining groups A and B shows that 88 per cent of the total of these

TABLE 3
CLASSIFICATION OF CASES ACCORDING TO THE BLOOD SUGAR-LOWERING RESPONSE TO THE BIGUANIDES

[illegible]

[illegible] the study These patients are not included in results unless they had side effects

TABLE 4
RESULTS WITH 300 CASES RECEIVING BIGUANIDES

	No	Per cent
Group A (*Successful*)	137	56
Group B (*Discontinued*)	79	32
Group C (*Failure*)	28	12
	244	100
Group D (*Deleted from study*)	56*	
Total	300	

* Includes 8 dropped from Group A because of no evidence of active diabetes

selected difficult patients demonstrated blood sugar lowering, while adding groups B and C shows that 44 per cent of the 244 cases either did not tolerate the drug or failed to show success in the dose used

Group D This group consists of the 56 patients who were deleted from the study group of 300 (TABLE 1)

Group C The 28 failures showed no pronounced characteristics They

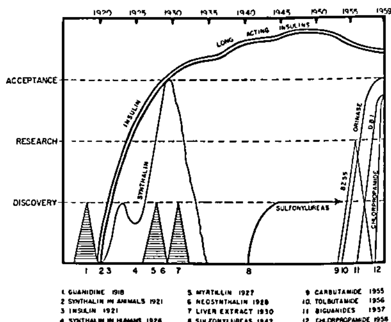


FIGURE 1 The rise and fall of the hypoglycemic agent dynasties.

TABLE 1

CLASS D 56 CASES DELETED FROM STUDY

28	Eye study only (subhypoglycemic doses)
17	Inconclusive
8	Inactive diabetes (shown by placebo studies)
3	Uncooperative
56	TOTAL

TABLE 2

DISTRIBUTION OF AGES AT ONSET OF STUDY

(244 Cases (Groups A, B, and C))

Ages	Cases
0 to 9	26
10 to 19	41
20 to 29	28
30 to 39	27
40 to 49	28
50 to 59	45
60 to 69	35
70 to 80	12
80+	2
Total	244

insulin daily with an average of 150 mg. of biguanide among total doses of 50 to 450 mg. daily. Seventy-five of these cases required no exogenous insulin supplement

Long-term studies. One hundred and seven of the 137 successful cases (group A) continue with biguanides. Forty-nine are taking supplemental, although

alone The

Seventy-

ith a range

of 12 to 28 months (average of this group, 22 months)

Toxicity studies. In the entire group at this time no toxicity has been manifested in liver function tests including Bromsulphalein retention, cephalin flocculation, thymol turbidity, alkaline phosphatase, or in the nonprotein nitrogen, white and red cell counts, hemoglobin, differential count, or complete urine analysis.

TABLE 6

COMPARISON AGE AT ONSET OF STUDY

(Total Study Versus Successful Group)

Ages	Groups A, B, and C	Group A	Net loss
0 to 9	26	18	−8
10 to 19	41	16	−25
20 to 29	28	12	−16
30 to 39	27	13	−14
40 to 49	28	22	−6
50 to 59	45	31	−14
60 to 69	35	21	−14
70 to 79	12	4	−8
80+	2	0	−2
	244 cases	137 cases	107 (total B + C)

Experimental Considerations

The biguanides offer interesting and ubiquitous contrasts They lower blood sugar levels in alloxanized and depancreatized animals as well as in dia-

l animals

cogen is not stored by the action of these compounds Despite these random observations, the mechanism of action is not yet known.

Recently Steiner and Williams[7] summarized their belief that DBI action is based on increased anaerobic glycolysis and suggested that this effect is due to inhibition of certain oxidative enzymes, namely, cytochrome oxidase and

complained of a "metallic taste" but not severely enough to discontinue the drug. The side effects were all gastrointestinal and were readily reversed by lowering the dosage or discontinuing the drug. Except for temporary discomfort, no other consequence resulted from these side effects. The best prevention was and still is to give the pills with meals and to use perceptive clinical acumen, adjusting the dose prophylactically when needed. The problem

TABLE 5
DURATION OF DIABETES AT ONSET OF STUDY*

137 cases—Class A successful ←		
1111111111 1111111111 1111111111 1111111111		1-1
2222222222 2222		
3333333		3
44444444		
5555555	Range 1 to 37 years, average 8 years	
66666666		6
7777		777
8888		8
999		9
10		
11-11		11
12-12-12-12		12-12
13-13-13		13-13
14-14-14-14		14
15-15-15		15
16-16		16
17-17		18
19		19
20		20
21-21		21
22		
23-23		
25-25	Compared with	25
26	28 cases—class B	
27-27	failures	27
28-28-28	→	28
29-29	Range 1 to 33 years	30
33	Average 14 6 years	32
35		33
37		

* Each number represents a separate case and indicates the actual duration in years of known diabetes

of side effects may be the greatest deterrent to widespread use of these drugs if not judiciously prescribed

Group A. According to previously cited criteria, 137 cases were classified as "successful" Comparing the ages of this successful group (A) with the total study group (A + B + C) in TABLE 6 shows that the attrition from the study in each age
the age 10 to 19 t
previous insulin.
with doses of 4 to 100 units.

After therapy was initiated, 62 patients used a daily average of 21 units of

insulin daily with an average of 150 mg of biguanide among total doses of 50 to 450 mg daily Seventy-five of these cases required no exogenous insulin supplement

Long-term studies One hundred and seven of the 137 successful cases (group A) continue with biguanides Forty-nine are taking supplemental, although reduced, doses of insulin, while 58 are treated with the tablets alone The duration of therapy is 3 to 28 months, with an average of 16 months Seventy-five cases have continued DBI or DBB for 12 or more months, with a range of 12 to 28 months (average of this group, 22 months)

Toxicity studies In the entire group at this time no toxicity has been manifested in liver function tests including Bromsulphalein retention, cephalin flocculation, thymol turbidity, alkaline phosphatase, or in the nonprotein nitrogen, white and red cell counts, hemoglobin, differential count, or complete urine analysis

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70 to 79	12	4	−8
80+	2	0	−2
	244 cases	137 cases	107 (total B + C)

Experimental Considerations

The biguanides offer interesting and ubiquitous contrasts They lower blood sugar levels in alloxanized and depancreatized animals as well as in diabetic humans There is considerable doubt that the depancreatized animals could exist for any length of time with DBI alone, and Fajans[6] observed that DBI-induced hypoglycemia in diabetics but not in normal human subjects Blood glucose levels are lowered and the glucose apparently utilized, but glycogen is not stored by the action of these compounds Despite these random observations, the mechanism of action is not yet known

Recently Steiner and Williams[7] summarized their belief that DBI action is based on increased anaerobic glycolysis and suggested that this effect is due to inhibition of certain oxidative enzymes, namely, cytochrome oxidase and succinic dehydrogenase

Tranquada *et al*[8] found no decrease in oxygen consumption in the liver during the hypoglycemic action of DBI, and no decrease in oxygen consumption or "availability" has been demonstrated in any tissue in vivo Bolinger *et al*[9] reported that DBI did not inhibit the deposition of glycogen by insulin in the

presence of adequate glucose. Fajans *et al.*[10] have shown that there is no change in the urinary excretion of amino acids (urinary nitrogen) when DBI is given to fasted diabetic patients Forbath and Clarke[11] stated that DBI does not increase intracellular glucose or glucose phosphorylation. These are essential steps in the initiation of anaerobic glycolysis. Even the relative blockade of Krebs cycle enzymes should induce hyperglycemia and not hypoglycemia.

The evidence for any definitive theory is yet not well delineated. The results thus far have been chiefly on animals, with dosage concentrations tremendously greater than those used in humans. Furthermore, they do not exclude other possible systems or mechanisms Steiner and Williams[7] best stated the need for more thoughtful and objective evaluation as follows: "... the clinical trial is equally as important as the laboratory, so long as the overall benefits to the patient are objectively assessed in as many ways as possible."

Clinical Observations

It is evident that the biguanides are capable of lowering blood sugar levels in a wide range of diabetics including patients in whom other oral agents are ineffective However, aside from the obvious gambit of "doing more than the other pills and doing it better," DBI shows unique ability to cooperate with insulin, and in a number of patients this drug has provided better regulation with fewer severe reactions and a subsequent more normal life. These were of that important minority, the unstable diabetic who is not doing well with even large doses of insulin, often given by multiple injection (TABLES 7 and 8)

DBI and its analogues are not effective in the successful management of human patients in the absence of exogenous or endogenous insulin. The juvenile-onset diabetics, with few exceptions, require supplemental, often smaller, amounts of insulin Some cases were regulated with biguanide and diet alone for long periods but invariably required some insulin when their diabetes remission period terminated. Many adult-onset type diabetics can be regulated with diet and biguanide alone, but it is likely that some endogenous insulin is intact and in action.

Part of our earlier high side-effect incidence was due to the fact that the first attempts were to replace insulin completely. In the past year the goal has been to stabilize and regulate without primary regard for the size of the insulin dose However, a high incidence of side effects is still possible, and until stabilization of dose is achieved in the more difficult diabetics, these patients must be carefully observed. In such cases the biguanides are a tactical rather than an operational weapon in the diabetic armamentarium.

In spite of the problems with the technique of regulation, no evidence of toxicity has yet been shown by liver, kidney, or hematological studies in several thousand patients Some of these have been under observation for almost three years

The incidence of severe hypoglycemic reactions in the well-regulated patient has markedly diminished with no hypoglycemic unconsciousness nor convulsive seizure in the entire study series (300 patients). There were many minor warning hypoglycemic episodes, however.

Further extensive experimental and clinical studies are needed. The analogue biguanides such as *n*-amyl (DBB) and *n*-butyl (DBV) appear to be better tolerated and are apparently superior to the phenethyl biguanide (DBI) in many patients. Joint administration of biguanide and sulfonylurea recently reported by Beaser[15] and Dolger[16] is based on the rationale that since *the sulfonylurea drugs may stimulate the pancreas to produce insulin, and the*

TABLE 7

Case No 359 (H D) Age 35 Duration diabetes 22 years
Usual insulin 90 units daily Diet 2100 calories

	Hosp day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Venous blood sugar values in mg per 100 cc (Somogyi Nelson)													
Fasting		105	240	74	270	167	179	129	126	152	215*	39	34	101
11 A M		161	189	93	261	114	137	40	108	58	125	84	95	
3 P M	65		127	46	243	95	166	78	67	87	69	60		
Units insulin	90	60	64	67	67	56	43	60	42	48	48	50	44	40
DBB (mg)						200	200	250	250	250	250	250	250	250

	Discharge medication			
	Breakfast	Lunch	Dinner	Bedtime
DBB mg	100	50	50	50
Insulin units				
NPH	24	0	0	10
Regular	6	0	0	0

* Insufficient bedtime insulin given

TABLE 8

CASE NO 227 AGE, 10 YEARS, INSULIN 38 U DURATION 2 YEARS, DIET 144 86 82

Hospital days	Capillary blood sugar			Insulin unit	DBI mg
	A M	Noon	P M		
Insulin + weighed diet					
1	—	—	254 mg %	38	
2	136	235	69	40	
3	75	—	—	37	
4	161	178	168	39	
5	108	197	116	40	
Weight diet + insulin + DBI					
6	92	—	45	28 +	150
7	155	70	55	20 +	150
8	—	54	35	16 +	150
9	124	74	62	8 +	100
10	97	39	—	8 +	100

biguanides appear to enhance or amplify insulin action, the combined action should be synergistic with smaller amounts of each In our own series this effect has been demonstrated in several cases (TABLE 9).

There has been considerable statistical attrition in this combined series of adults and juveniles, as shown in FIGURE 2. Although 137 cases were classi-

TABLE 9
CASE NO 221 AGE, 48 YEARS INSULIN 30 U. DURATION 8 YEARS WEIGHED DIET*

Hospital days	Blood sugar 7 A M	11 A M	3 P M	Insulin units	DBE gm	DBI gm
Insulin						
1	—	—	96 mg %	30		
2	137	226	—	26		
3	88	159	109	30		
4	—	304	—	30		
DBE						
5	62	—	—		1 0	
6	76	100	—		1 0	
7	118	209	207		0.75	
DBE + DBI						
8	126	207	108		1 0 +	150
9	114	—	95		0.5 +	.150
10	94	—	123		0 5 +	150
11	71	110	124		0 5 +	200
12	94	—	107		0 25 +	.200
13	102	141	103		0 25 +	.150

* In this case all blood sugar determinations are with the Somogyi-Nelson method The 7 A M blood samples are venous, while the 11 A M and 3 P M samples are capillary The DBI used was *n*-amylbiguanide, DBE represents chlorpropamide (Diabinese)

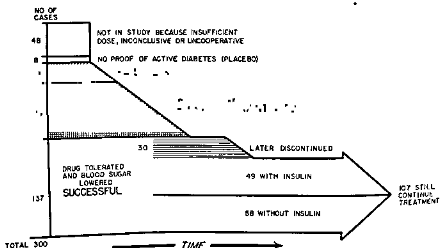


FIGURE 2 Statistical attrition in 300 diabetics receiving biguanides (including severe and juvenile onset cases)

fied as "successful," 30 of these were later discontinued from therapy for the following reasons uncooperative, 12, unrelated complications, 6 (arterial graft, 1, chronic lymphocytic leukemia, 1, pregnancy, 1; coronary occlusion, 2, dead from myocardial infarction, 1) There were 12 other cases that were well regulated for varying periods of time but that were then discontinued because of later failure or because of a loss of well-being and tiredness that sometimes persisted in the face of normal blood sugar values.

Fifty-nine of the 300 study cases failed to respond to one of the sulfonylurea group either by trial or test When given DBI or DBB, their response was. (A) successful, 34, (B) discontinued, 14, (C) failure, 5, and (D) deleted, 6

Discussion

None of the presently available oral hypoglycemic agents is completely adequate, and all seem to nibble at the periphery of lower blood sugar values rather than strike at the basic aspects of diabetes. There is even some question as to whether or not the diabetes or the blood sugar level is being treated. On the other hand, contemporary forms of insulin sometimes have shortcomings, particularly in the therapy of the brittle, long-term diabetic Many patients alternately fluctuate between quasiacidosis and convulsive hypoglycemia Present types of insulin force the physician to commit himself 24 hours in advance, estimating the dietary intake and physical and metabolic activity as well as sudden onset of illness or accident Insulin, however, is more physiological, and this fact has been proved over the years Here, problems arise not so much from insulin per se as from the present forms of insulin

There is no royal road to diabetes regulation and treatment and no shibboleth that is an easy pass-word to successful therapy Diabetes is an individualistic problem, and no mass-produced treatment to date can solve all the facets Each of the present components, diet, insulin, sulfonylureas and biguanides, is a tool to be used, singly or in combination, for a specific situation (FIGURE 3) These tools are of different weight and varying importance The present age is one of specialization in all fields of endeavor This is the era of the precise tool, in which careful techniques and special tools are used to achieve desired results In diabetes these tools should be aimed at a more careful and precise regulation As understanding increases and more tools are available, second-best and compromise therapy should be less tolerated than ever before The era of the precise tool must not, for physicians, degenerate into an age of convenience, with an acceptance of lesser standards of treatment because of the convenience of oral therapy

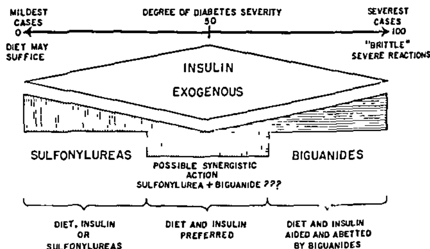
Summary

(1) A selected group of 244 patients including many difficult, unstable diabetics was treated for periods up to 28 months with the phenethyl (DBI) and n-amyl (DBB) biguanides

(2) Eighty-eight per cent of this group responded with significant blood sugar lowering, while 44 per cent (32 per cent side effects, 12 per cent failure) of the total discontinued treatment because of side effects or failure to demonstrate blood sugar lowering in the doses used

(4) While gastrointestinal side effects were present in a significant number of patients, they were reversed by lowering the dosage or discontinuing the drug

(5) No evidence of toxicity occurred with serial tests of hepatic, renal and hematological function.



(6) Significant convulsive hypoglycemic reactions or resulting unconsciousness were not found.

(7) The exact mechanism of action is still a moot point, but probably the biguanides depend on exogenous or endogenous insulin for their effectiveness. No total juvenile-onset or severe, brittle diabetic has been successfully regulated without supplemental insulin for any considerable length of time

(8) The biguanides, when carefully employed, in this series have been useful adjuncts in the stabilization of unstable and brittle diabetes.

(9) None of the oral agents is completely adequate However, diet, the present forms of insulin, the sulfonylureas, and the biguanides are to be considered

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oral hypoglycemic agent, Phenformin (DBI) Baylor Univ Coll Med Houston, Texas

Med Houston, Texas

METAHEXAMIDE IN DIABETES THERAPY

James M Moss and DeWitt DeLawter
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The search for the most potent and safest drug to be used in the oral treatment of diabetes has been continuous since the sulfonylurea drugs were first found to have hypoglycemic properties in 1942. Tolbutamide is the most widely used drug and its efficiency and safety has been attested by many reports[1] It has been used in more than 400,000 patients with a prolonged satisfactory therapeutic response in more than one half of the cases and with minimal toxicity. In our own series of 200 patients, we obtained initial improvement in 84 per cent[2] After 1 year of treatment this was reduced to 54 5 per cent,[3] and after 2 years of treatment, to 46 5 per cent.[4] This gives an over-all secondary failure rate of 38 5 per cent or about 3 per cent of those under treatment each month. This high rate of secondary failure is offset by a lower rate of primary failure than has been reported by others[1] This difference is due to the fact that we continued to use the drug in 67 patients with initial poor or fair results. Our 46.5 per cent successful patients at the end of 2 years is comparable with the 53 4 per cent reported by Mehnert *et al.*[5] at the end of 20 months if all of their unselected patients are included as ours were

Chlorpropamide was introduced because the same hypoglycemic effect can be obtained with a smaller dosage.[6] In our experience with 46 patients the comparable dose is about one third to one fourth that of tolbutamide, apparently due to a higher blood level resulting from the slower excretion In general there is no significant difference in the results obtained with the two drugs. A few patients did show better results, but this can often be explained by the fact that it was easier for the patient to remember to take 2 or 3 tablets of chlorpropamide each morning than to try to remember to take 1 or 2 tablets of tolbutamide 2 to 4 times each day When the drug is given in adequate doses any patient who does not respond well to one compound will not do well on the other Since the dosage we used did not exceed 1 gm /day we did not encounter any patients with jaundice or other evidence of liver damage There was one patient who had an aggravation of her anginal syndrome after being changed from tolbutamide to chlorpropamide, but this improved after the dose was reduced and the blood sugar allowed to rise.

Metahexamide has been used in 70 patients with excellent results in 31, good results in 14, fair results in 2, and poor results in 23 (only 51 patients are shown in FIGURE 1) There were 32 patients who had been treated with tolbutamide or chlorpropamide previously Of the 14 with satisfactory results in the past there were 9 excellent, 4 good, and 1 fair response. Of the 18 with unsatisfactory results in the past, there were 14 failures, 2 poor, and 2 fair response These last four patients either had been secondary failures or p previously. They have not been followed long enough for cor 'on It can be seen that these results are similar to those other sulfonylurea drugs and that metahexamide will do mide will not do if an adequate dose is given

We found that the private patients usually did better than the clinic patients. Much of this difference can be explained by the fact that the private patients were more careful about dietary control and the avoidance of obesity [illegible] response the dose was raised to 400 and occasionally to 600 mg before the drug was discontinued In 21 patients who took 400 mg or more for 1 to 30 days, there were 4 patients with features of acute gastritis characterized by epigastric

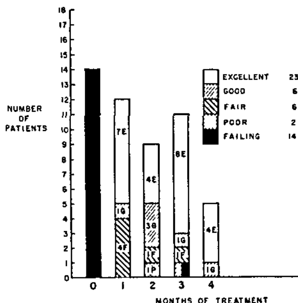


FIGURE 1 Results of metahexamide treatment in 51 patients

hunger pains, nausea, and vomiting These symptoms cleared promptly after the drug was discontinued No liver function tests were conducted on the patients taking high doses Alkaline phosphatase determination made on 20 patients on the usual maintenance dosage have shown no significant elevation

One patient developed massive gastrointestinal hemorrhage after taking 200 mg for a period of 2 weeks with excellent diabetic control Complete gastrointestinal X-ray examinations were normal Prothrombin time and blood clotting were normal She had a similar episode 3 years ago with no apparent cause and there never have been any symptoms of gastrointestinal disease Six pints of blood were required to raise the hematocrit from 22 to 42 She is now well controlled on 1 gm of tolbutamide each day.

Another patient died of myocardial infarction after taking 100 mg /day for 2 days There is no evidence that the drug was a factor in his death.

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Diabetic Clinic, Georgetown University Medical Center, Washington, D C.

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Twenty-one patients were given placebo tablets for 1 to 4 weeks before being placed on metahexamide. Almost all of these patients showed a fall in blood sugar during the first week or two of treatment followed by a rise as the novelty effect of the new medication wore off. This placebo effect explains some of the secondary failures that had been reported from other sulfonylurea drugs. FIGURES 2 and 3 show that the patients who obtained the best response to the placebo also obtained the best response to metahexamide. This can be explained by the fact that the patients with the more severe diabetes did not respond well to either. There were two patients who had been on 10 to 15 U. of insulin each day who were better controlled by the placebo than by metahexamide or tolbutamide. There have been others in whom the diabetes went out of control when the placebo was used, but who responded well to a sulfonylurea drug. Placebo studies are essential for the proper evaluation of any oral hypoglycemic drug and such studies should be done by the double-blind method.[7]

Summary and Conclusions

(1) The results obtained from metahexamide in a series of 70 patients are similar to those obtained from tolbutamide or chlorpropamide.

(2) Toxic effects of nausea, vomiting and epigastric pain were seen in one

studies essential for the proper evaluation of any hypoglycemic drug.

(5) The only apparent advantage of metahexamide is the small size of the pill.

Addendum

is our opinion that further use of this drug should be discontinued.

References

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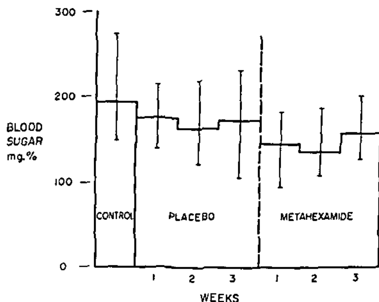


FIGURE 2 Comparative effect of placebo and metahexamide in eleven patients with a satisfactory response to metahexamide

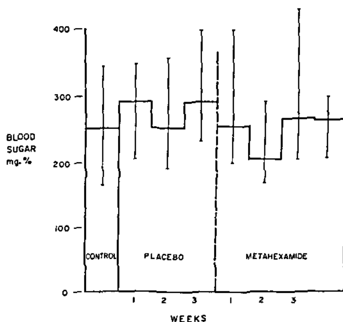


FIGURE 3 Comparative effect of placebo and metahexamide in seven patients with primary failure to metahexamide

Twenty-one patients were given placebo tablets for 1 to 4 weeks before being placed on metahexamide. Almost all of these patients showed a fall in blood sugar during the first week or two of treatment followed by a rise as the novelty effect of the new medication wore off. This placebo effect explains some of the secondary failures that had been reported from other sulfonylurea drugs. FIGURES 2 and 3 show that the patients who obtained the best response to the placebo also obtained the best response to metahexamide. This can be explained by the fact that the patients with the more severe diabetes did not respond well to either. There were two patients who had been on 10 to 15 U. of insulin each day who were better controlled by the placebo than by metahexamide or tolbutamide. There have been others in whom the diabetes went out of control when the placebo was used, but who responded well

Summary and Conclusions

(1) The results obtained from metahexamide in a series of 70 patients are similar to those obtained from tolbutamide or chlorpropamide.

(2) Toxic effects of nausea, vomiting, and epigastric pain were seen in one fifth of the patients who took 400 mg. or more per day.

(3) The average maintenance dose is 150 mg./day and there is no value in exceeding a dose of 300 mg./day.

(4) Beneficial effects following placebo administration makes double-blind studies essential for the proper evaluation of any hypoglycemic drug.

(5) The only apparent advantage of metahexamide is the small size of the pill.

Addendum

is our opinion that further use of this drug should be discontinued.

References

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7. HURWITZ, D. & A. C. MCCUISTON. 1957. Tolbutamide: a double-blind study of its effect in diabetes. New Engl. J. Med. 257: 931–933.

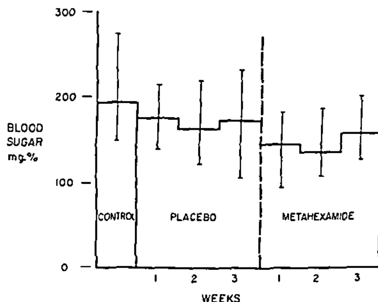


FIGURE 2 Comparative effect of placebo and metahexamide in eleven patients with a satisfactory response to metahexamide.

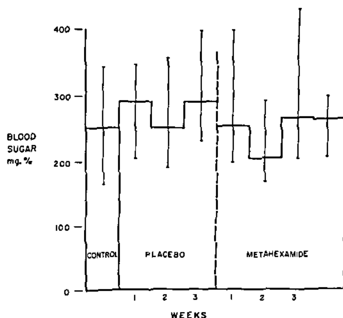


FIGURE 3 Comparative effect of placebo and metahexamide in seven patients with primary failure to metahexamide

better control

In a similar manner the patients not hospitalized were given the drug and requested to test the urine at home before every dose of metahexamide Those taking insulin were given metahexamide concomitantly Usually the dose of insulin was lowered by decrements of 10 or 15 U as control was maintained by a constant dose of metahexamide, until eventually no insulin was needed or an optimum combination of insulin and metahexamide was obtained If the

TABLE 1
EFFECT OF METAHEXAMIDE ON INSULIN REQUIREMENTS

Patient	Age	Duration of diabetes (years)	Average insulin requirements		Average blood sugar		Dose of metahexamide (mg /day)
			Before	After	Before	After	
1	56	4	40	0	136	140	150
2	57	1	15	0	123	170	50
3	50	4	20	0	230	120	100
4	55	15	20	0	294	166	100
5	61	12	10	0	179	192	100
6	43	9	30	15	256	191	100
7	65	6	35	0	127	153	50
8	72	9	50	0	84	187	100
9	52	1/12	30	0	216	131	100
10	53	7	40	0	179	144	100
11*	39	4	10	0	142	144	100
12	76	2	10	0	242	178	100

* Previous secondary failure with tolbutamide

factory, thereafter the frequency of visits was decreased as improvement progressed

Those patients taking tolbutamide or chlorpropamide, but with persistent glycosuria and fasting blood sugar always higher than 200 mg /100 ml , were considered not adequately managed They were placed directly on metahexamide and followed in the same manner as described above

Several patients were only partly controlled by diet alone They were given metahexamide in the same starting dosage as described above, and the same type of follow-up was observed

Results

The results of therapy with metahexamide in the 3 groups of patients are presented in TABLES 1, 2, and 3 A total of 35 patients have been followed for 1 to 4 months Of these, 22 (63 per cent) showed adequate control either on metahexamide alone or in combination with insulin at a reduced dose

FIGURE 1 illustrates the good response of a patient to metahexamide. He

CLINICAL STUDIES WITH METAHEXAMIDE

Samuel J. N. Sugar and Lawrence J. Thomas
George Washington University School of Medicine, Washington, D. C

The investigation described below was conducted to confirm the antidia-

as 4 months

The study was done in the Diabetic Clinic, District of Columbia General Hospital, George Washington University Division, Washington, D. C, and the Medical Department of Prince George's General Hospital, Cheverly, Md.

Method of Study

Diabetic patients who were taking insulin, those inadequately controlled with tolbutamide or chlorpropamide, and patients only partly controlled by diet alone were studied Some patients were hospitalized and others were observed in the outpatient clinics and in private practice. In the hospitalized patients, levels of sugar in the blood (fasting and post prandial) and in the urine (before meals and at bedtime) were determined. Ketosis, if present, was relieved by regular insulin injections and parenteral fluid Later a measured diet was prescribed and control maintained by doses of regular insulin given fractionally according to urinalyses. The insulin dose ranged from 5 to 25 U , depending on the amount of glucose and acetone present Metahexamide was then added to the regime, usually in a dose of 50 mg. three times daily before meals If control with metahexamide was effective it was usually manifested in a few days by decrease in insulin requirement, reduction in blood and urine sugar levels, and by an increase in the feeling of well-being of the patient. If control was not attained at this dose level, metahexamide was increased by 50-mg increments until a satisfactory response was noted If metahexamide seemed ineffective after a 7-day trial, it was discontinued and insulin dosage adjusted for control.

Management with metahexamide was considered satisfactory if the average daily fasting blood sugar was substantially reduced and was in the region of 175 mg /100 ml or below. A corresponding lowering of urinary glucose was required, the ultimate goal being complete aglycosuria When at least 3 days' observation indicated these requirements had been attained, patients were given a supply of medication and discharged, to be followed in the clinic or office. Particular stress was laid on the need for frequent urine tests at home, and a written record of the results was requested On the return visit, the patient was examined, home urine tests reviewed, and the fasting blood sugar and urinalysis for that day determined If most of the urine tests were reported negative and if the blood sugar was 120 mg /100 ml or less, the dose of metahexamide was reduced by 50 mg If the blood sugar was between 120

* The metahexamide used in the study reported in this paper was supplied by C J O'Donovan of The Upjohn Company, Kalamazoo, Mich

had been taking an average of 40 U of insulin daily for 7 years Transfer to metahexamide was accomplished smoothly and without incident FIGURE 2 illustrates improved control with metahexamide in a patient not adequately managed with chlorpropamide

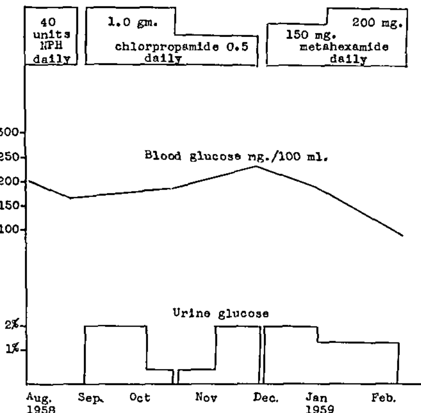


FIGURE 2 Fair control with metahexamide after failure with chlorpropamide

A high degree of success (100 per cent of 7 cases) was noted with metahexamide given to patients only partly controlled by diet alone The same good results with tolbutamide and chlorpropamide in this type of patient was reported previously by us [1,2]

A summary of the effectiveness of metahexamide in the different classes of diabetics is shown in TABLE 4

TABLE 2

PATIENTS RESPONDING TO METAHEXAMIDE AFTER SECONDARY FAILURE WITH TOLBUTAMIDE OR CHLORPROPAMIDE

Patient	Age	Duration of diabetes (years)	Dose (mg /day)		Average blood sugar		Dose of metahexamide (mg /day)
			Tolbutamide	Chlorpropamide	Before metahexamide	After metahexamide	
1	54	4	2000		240	193	200
2	58	8		500	228	108	200
3	62	9	1000		307	217	200
4*	30	1		750	236	178	100

* After 2 months control with metahexamide it was necessary to place this patient on insulin

TABLE 3

PATIENTS SATISFACTORILY MANAGED WITH METAHEXAMIDE WHO WERE NOT PREVIOUSLY CONTROLLED WITH DIET ALONE

Patient	Age	Duration of diabetes (years)	Dose of metahexamide (mg /day)	Blood sugar under		Duration of treatment (months)
				Diet	Metahexamide	
1	75	5	50	254	150	3
2	50	4	300	256	159	2
3	38	3	200	200	183	3
4	51	1/12	150	288	127	1
5	53	3	150	238	148	1
6	42	1/12	50	202	78	5
7	75	6	100	307	157	2

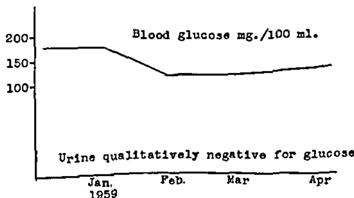


FIGURE 1 Good control with metahexamide after discontinuing insulin

had been taking an average of 40 U. of insulin daily for 7 years. Transfer to metahexamide was accomplished smoothly and without incident. FIGURE 2 illustrates improved control with metahexamide in a patient not adequately managed with chlorpropamide.

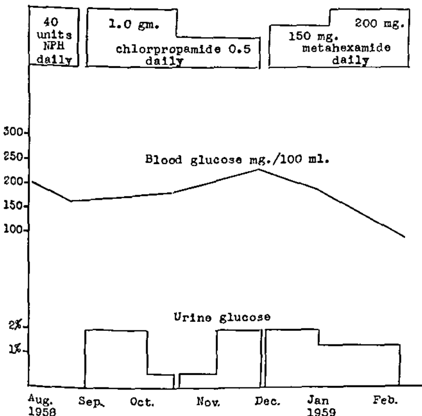


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				Diet	Metahexamide	
1	75	5	50	254	150	3
2	50	4	300	256	159	2
3	38	3	200	200	183	3
4	51	1/12	150	268	127	1
5	53	3	150	238	148	1
6	42	1/12	50	202	78	5
7	75	6	100	307	157	2

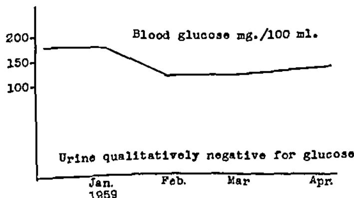


FIGURE 1. Good control with metahexamide after discontinuing insulin

Side effects of metahexamide in doses ranging from 50 to 300 mg a day were minimal.

Acknowledgment

We are grateful to C J Burns and his laboratory staff for their assistance in performing laboratory toxicity studies

References

Toxicity

The usual precautionary laboratory studies (serial blood counts and urine examinations, and liver and kidney function tests) were made on hospitalized patients With a daily dose range of 50 to 300 mg. metahexamide, no unusual bone marrow, liver, kidney, or thyroid effects have been noted. In fact, 1 case of acute cholangiolitic hepatitis complicating diabetes was adequately maintained on metahexamide after the diabetes was brought under control with insulin The side effects noted (TABLE 5) were not severe Only 1 patient noted hypoglycemic symptoms 10 days after starting therapy; these cleared promptly after eating. Two patients complained of epigastric burning, nausea, and vomiting. Generalized muscular weakness and vertigo were subjective complaints in 2 cases One patient noted drowsiness and another itching without rash.

TABLE 4
RESULTS OF TREATMENT

	23	
	11	
	1	
	12	(52%)
Number of patients treated who had previous tolbutamide or chlorpropamide failure	5	
Satisfactory control with metahexamide	3	
Number of patients not controlled on diet alone	7	
Satisfactory control with metahexamide	7	(100%)
Total number of patients treated	35	
Satisfactory control with metahexamide	22	(63%)

TABLE 5
SIDE REACTIONS TO METAHEXAMIDE

	No of patients*
Nausea and vomiting	2
Weakness	2
Dizziness	2
Itching	1
Drowsiness	1

* Of a total number of 35 treated

Conclusions

control with tolbutamide or chlorpropamide

19 hours
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e marked

isparity of our results permit no conclusions to be drawn

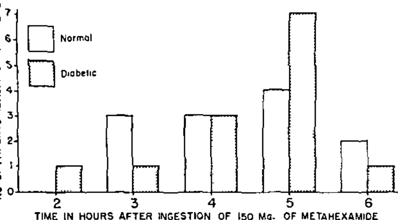


FIGURE 1 The distribution of appearance time for peak serum levels of metahexamide, in fasting diabetic and nondiabetic subjects, after the ingestion of 150 mg of metahexamide

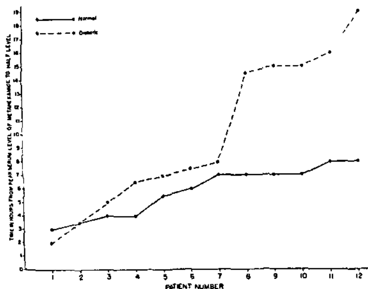


FIGURE 2 The time for the serum metahexamide level to drop to one half of its peak level in diabetic and nondiabetic subjects.

LABORATORY AND CLINICAL OBSERVATIONS WITH A NEW SULFONYLUREA

A. A Silver, H. Wishinsky, R. F. Caplan, C. Monk

Division of Biochemistry, Departments of Medicine and Pathology, Sinai Hospital of Baltimore, Inc., Baltimore, Md.

This report describes our clinical and laboratory experience with 3-amino-4-methylbenzenesulfonylcyclohexylurea (metahexamide). This drug differs from other effective oral antidiabetic sulfonylureas in that this compound has been reported to combine therapeutic effectiveness with serum levels of long duration [1]

Methods and Results

Reviewing the Boehringer Reports[1] on WP-40 (metahexamide) prior to our study, we observed that when the authors used single doses of 500 mg. and 1 gm. of metahexamide, they found half-value periods of 20 and 22 5 hours, respectively We chose to use daily doses of 150 mg of metahexamide, the recommended safe therapeutic dose,* in our studies. Half-value studies or the disappearance rate of metahexamide from the serum were studied, using the 150-mg dose in order to relate these values to the previously mentioned 500 mg and 1 gm doses. Thirteen diabetic and 12 nondiabetic patients that had not previously received metahexamide were given, while fasting, a single oral dose of 150 mg of the drug. Blood specimens were drawn prior to drug therapy and then 1, 2, 3, 5 7, 9, 12, and 24 hours after ingesting the metahexamide. Serum levels of metahexamide were determined using the method of Bratton and Marshall [2] FIGURE 1 shows the number of hours after ingestion of the drug for appearance of peak serum levels of metahexamide FIGURE 2 shows the number of hours required for the peak level of metahexamide in the

[illegible]

by the Boehringer group

FIGURE 3 represents the percentage of metahexamide remaining in the serum 19 hours after peak level of the drug The choice of 19 hours after peak level was one of expediency. Percentages were calculated by dividing the serum concentration of metahexamide 19 hours after peak level by its concentration at peak level. It is apparent that the nondiabetics as a group demonstrate lower concentrations of serum metahexamide 19 hours after peak level than the di[illegible]

shown [illegible]

showing [illegible]

P indicate those patients who later, on continuous therapy, showed (*G*) good clinical control and (*P*) poor clinical control. It is obvious that no conclusion can be drawn from the single dose test in determining the value of this compound in the treatment of any given patient, with our limited number of pa-

* Recommended by C J. O'Donovan, The Upjohn Company, Kalamazoo, Mich

that they might benefit from this compound Several patients on previous good control with insulin who requested a trial with an oral compound are also included In all cases we began the study with a decrease in the chemical control by reducing the insulin or substituting a placebo for the previous oral compound until there was a rise in the blood sugar level and/or the appearance of glycosuria Diet was unchanged from the pretreatment value

TABLE 1
PATIENT CONSTANCY OF SERUM LEVELS OF METAHEXAMIDE

M R *,§		D E **,‡		I N *,‡		A S *,§		R C **,‡		D R †,‡	
days	mg %	days	mg %	days	mg %	days	mg %	days	mg %	days	mg %
1	1 0	1	0 2	1	0 9	1	1 1	1	1 1	1	0 4
4	1 4	4	0 2	7	0 8	13	1 2	18	1 5	40	0 2
14	1 4	14	0 2	19	0 5	34	1 5	25	1 6	55	0 3
22	1 5	22	0 2	26	0 6	46	1 0	38	1 7	62	0 4
28	1 4	28	0 2	40	0 7	63	1 2	45	1 6	77	0 4
35	1 1	35	0 2	54	0 9			50	1 2		
42	1 1	42	0 2					61	1 4		
47	1 0							67	1 5		
64	1 2							82	1 2		
81	0 9										

Key (*) daily dose of 150 mg , (**) daily dose of 200 mg , (†) daily dose of 100 mg , (‡) daily predrug serum levels, and (§) serum drug levels 2 hours after drug ingestion

TABLE 2
PATIENT C K SERUM LEVELS OF METAHEXAMIDE

Single dose (150 mg)*		Daily dose (150 mg)†		
Predrug (hours)	mg % 0	Day	Predrug mg %	5 hrs later mg %
1	0 5	1	0 5	1 6
2	0 6	2	0 5	1 5
3	0 5	3	0 8	1 7
5	0 4	4	0 5	1 6
7	0 3	5	0 5	1 7
12	0 2			
24	0			

* Prior to treatment
† One month after treatment (daily dose 150 mg)

Forty-five diabetics previously well controlled with tolbutamide were equally well controlled with metahexamide They were transferred to metahexamide beginning with daily doses of 150 mg In most cases this dose could soon be reduced After several weeks of therapy, the amount of metahexamide employed was in the ratio of 5 to 10 mg /100 mg tolbutamide FIGURE 4 illustrates our results on H L , a 50-year-old white male, a diabetic of 1-year dura-

If metahexamide, as was *previously* stated, maintained serum levels of long duration, the possibility must be considered that patients on chronic therapy might increase their blood drug levels slowly. Serum metahexamide and blood sugar levels were frequently *determined during* the course of the chronic studies Blood samples were always drawn at a given time in relation to the ingestion of the metahexamide. TABLE 1 shows a remarkable individual constancy of serum metahexamide level *in 6* patients studied for periods up to 2½ months. TABLE 2 is interesting in that patient C. K. and several *others in the initial single-dose test 19 hours after peak level* had zero or negligible

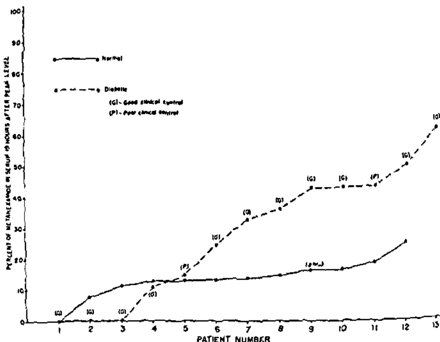


FIGURE 3. The percentage of *maximum* metahexamide in subject's serum 19 hours after peak serum level of the drug

serum metahexamide levels *Continued* therapy yielded detectable fasting

1

it *there* is no cumulative effect of

h metahexamide by oral administration The initial treatment in all cases was *150 mg.* given as a single fasting dose. Patients were selected chiefly on the basis of *previous* good response to tolbutamide. A few patients were selected because of the previous poor response to oral antidiabetic drugs and poor response to *insulin*, in the hope

that they might benefit from this compound Several patients on previous good control with insulin who requested a trial with an oral compound are also included In all cases we began the study with a decrease in the chemical control by reducing the insulin or substituting a placebo for the previous oral compound until there was a rise in the blood sugar level and/or the appearance of glycosuria Diet was unchanged from the pretreatment value

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M R *,‡		D E **,‡		I N *,‡		A S * §		R C ** §		D R †,‡	
days	mg %	days	mg %	days	mg %	days	mg %	days	mg %	days	mg %
1	1 0	1	0 2	1	0 9	1	1 1	1	1 1	1	0 4
4	1 4	4	0 2	7	0 8	13	1 2	18	1 5	40	0 2
14	1 4	14	0 2	19	0 5	34	1 5	25	1 6	55	0 3
22	1 5	22	0 2	26	0 6	46	1 0	38	1 7	62	0 4
28	1 4	28	0 2	40	0 7	63	1 2	45	1 6	77	0 4
35	1 1	35	0 2	54	0 9			50	1 2		
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47	1 0							67	1 5		
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Single dose (150 mg)*		Daily dose (150 mg)†		
Predrug (hours)	mg % 0	Day	Predrug mg %	5 hrs later mg %
1	0 5	1	0 5	1 6
2	0 6	2	0 5	1 5
3	0 5	3	0 8	1 7
5	0 4	4	0 5	1 6
7	0 3	5	0 5	1 7
12	0 2			
24	0			

* Prior to treatment
† One month after treatment (daily dose 150 mg)

Forty-five diabetics previously well controlled with tolbutamide were equally well controlled with metahexamide They were transferred to metahexamide beginning with daily doses of 150 mg In most cases this dose could soon be

when he was abruptly transferred to metahexamide with no disturbance in control After 10 days his dose was reduced to 100 mg; on the fiftieth day it was further reduced to 50 mg. daily, with which he continues to be well maintained

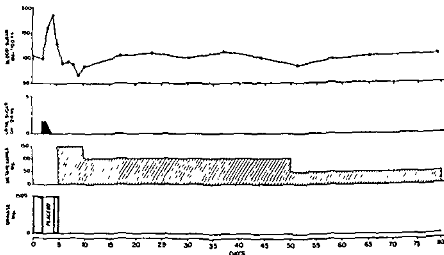


FIGURE 4 H L, male, age 52 years, diabetic one-year duration Previously well controlled with tolbutamide Good control with metahexamide

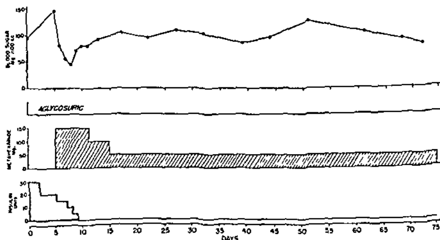


FIGURE 5 K. H, female, age 72 years, diabetes 5-years duration. Previously well controlled with insulin. Good control with metahexamide

Eleven of 18 patients previously well controlled by insulin were transferred to metahexamide within 30 days or less. The remaining 7 of this group are still taking insulin in doses varying from 30 to 75 per cent of their intial amount. FIGURE 5 illustrates the transfer of K. H, a patient well controlled with diet

and insulin, to metahexamide. This 72-year-old white female, a diabetic of 5-years duration, requested transfer to an oral agent because of difficulty in self administration of insulin. Her daily dose of insulin was first reduced to produce hyperglycemia and then increased until the fasting blood sugar was satisfactory An initial dose of 150 mg metahexamide was given and maintained until insulin was discontinued Generally, we reduced insulin very slowly, rarely over 15 per cent in any given day In this case insulin was reduced rapidly by our standards The dose of metahexamide was gradually reduced until she is now maintained on 50 mg daily in good control FIGURE 6 shows the results with L N, a 61-year-old white female, a diabetic of 12-

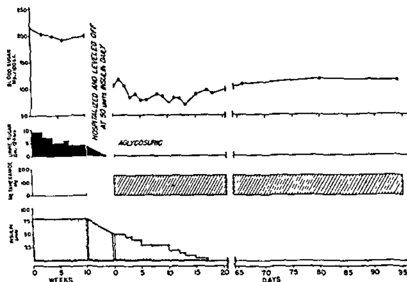


FIGURE 6 L N, female, age 61 years, diabetes 12-years duration Previous treatment insulin, well controlled with metahexamide

years duration, transferred from insulin to metahexamide This patient did not have good control with insulin, although we suspect she now follows her diet much better than prior to this transfer Although her daily insulin requirement was 80 U, under close hospital supervision she leveled off at 50 U. The initial dose was 150 mg of metahexamide and insulin was gradually reduced to zero FIGURE 7 illustrates another patient, S B, a 66-year-old white female, a diabetic of 16-years duration, previously maintained on diet and insulin, and now transferred to metahexamide. In this case we decided to try a greater percentage decrease in insulin An abrupt rise in blood sugar necessitated an increase in insulin, 25 per cent above the initial dose. How-

when he was al
control After
was further redu
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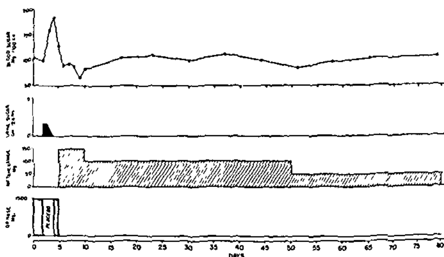


FIGURE 4 H L, male, age 52 years, diabetic one-year duration Previously well controlled with tolbutamide Good control with metahexamide.

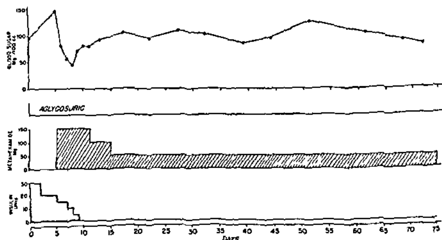


FIGURE 5 K H, female, age 72 years, diabetes 5-years duration Previously well controlled with insulin Good control with metahexamide

Eleven of 18 patients previously well controlled by insulin were transferred

FIGURE 6

400 mg. daily. He showed evidence of control for a short period, then quickly reverted to the unstable state Metahexamide was discontinued after 12 weeks

Four of the 67 patients treated with metahexamide were taken off the drug, 1 for obvious failure, as stated above, and the remaining 3 because of side effects

Toxicity

The following laboratory studies were done on all cases prior to and during therapy serum transaminase, thymol turbidity, Bromsulphalein retention, alkaline phosphatase, blood urea, creatinine, urinalysis, phenolsulfonphthalein, complete blood count, and platelet count Protein-bound iodine tests were done in most cases All of these studies were repeated (except PBI) at weekly intervals for 4 weeks and then semimonthly up to 4 months

Three of the 67 patients treated with metahexamide were removed from treatment with the drug because of side effects. One developed severe urti- [illegible] peared with placebo but returned on daily doses of 50 mg metahexamide Laboratory tests were negative except in three patients who developed moderately elevated alkaline phosphatases Two of these three patients had elevated transaminases These patients show no clinical evidence of hepatic toxicity

Summary

Clinical and laboratory data have been presented and discussed on sixty-seven diabetic patients treated with metahexamide for periods up to forty weeks

levels in any given subject on constant daily doses (at constant time after ingestion of the drug), indicating absence of cumulative effect of metahexamide

Our findings indicate that patients who responded well to tolbutamide therapy can be transferred uneventfully to metahexamide

Acknowledgments

We thank the Upjohn Company, Kalamazoo, Mich., and Eli Lilly and Company, Indianapolis, Ind , for the metahexamide used in this study

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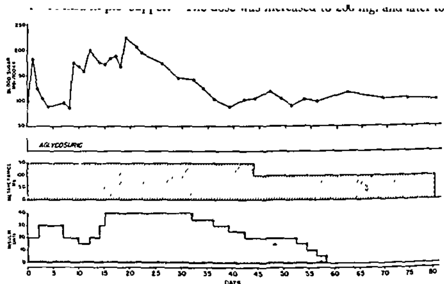


FIGURE 7 S. B., female, age 66 years, diabetes 16-years duration. Previously controlled with insulin. Good control with metahexamide.

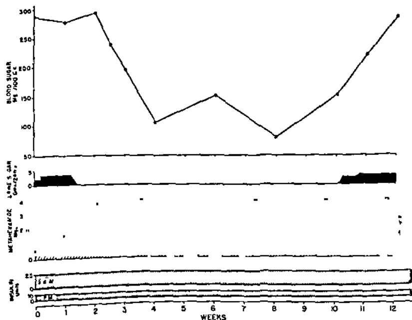


FIGURE 8 S. P., male, age 41 years, diabetes 5-years duration. Poorly controlled with insulin. Did not respond with metahexamide.

TABLE 1

CAPILLARY VENOUS (C-V) GLUCOSE DIFFERENCES IN DIABETIC MEN, DURING FASTING AND AFTER METAHEXAMIDE OR INSULIN

Medication	Case No	Blood glucose (mg /100 ml) Capillary Hour 0	1	2	3	4	5	Venous Hour 0	1	2	3	4	5	Differences 0	1	2	3	4	5
None	4	80	98	110	102	98	108	77	90	98	95	90	90	3	8	12	7	8	18
	7	193	199	189	178	176	168	192	194	189	182	176	169	1	5	0	−4	0	−1
	8	124	121	124	128	123	113	120	117	117	123	117	113	4	4	7	5	6	0
	10	187	186	180	181	172	155	187	187	181	179	171	150	0	−1	−1	2	1	5
	11	130	133	122	115	100	108	120	122	113	112	94	102	10	11	9	3	6	6
Totals		714	737	725	704	669	652	696	710	698	691	648	624	18	27	27	13	21	28
Averages		143	147	145	141	134	130	139	142	140	138	130	125	3.6	5.4	5.4	2.6	4.2	5.6
Metahexamide (500 mg)	4	108	99	101	79	71	80	77	78	76	60	63	66	31	21	25	19	8	14
	7	209	209	189	179	174	172	204	200	187	170	170	166	5	9	2	9	4	6
	8	100	75	72	70	72	90	90	72	70	60	66	66	10	3	2	10	6	4
	10	159	153	149	143	124	122	151	151	140	131	119	112	8	22	9	12	5	10
	11	130	122	115	108	93	92	103	103	94	93	90	78	27	19	21	15	3	14
Totals		706	658	626	579	534	536	625	604	567	514	508	488	81	54	59	65	26	48
Averages		141	132	125	116	107	107	125	121	113	103	102	97	16.2	11.0	11.8	13.0	5.2	9.6
Insulin 10 U	4	88	72	51	50	41	41	86	66	44	51	41	43	2	6	7	−1	0	−2
40 U	7	219	198	73	113	123	80	198	164	56	80	94	63	21	34	17	33	29	17
20 U	8	173	156	81	57	56	57	160	133	62	37	35	43	13	23	19	20	21	14
40 U	10	219	182	131	98	84	88	218	178	126	91	86	81	1	4	5	7	−2	7
20 U	11	144	107	57	59	55	55	136	102	51	51	56	50	8	5	6	8	−1	5
Totals		843	715	393	377	359	321	798	643	339	310	312	280	45	72	54	67	47	41
Averages		169	143	79	75	72	64	160	129	68	62	62	56	9.0	14.4	10.8	13.4	9.4	8.2

BLOOD LEVELS FOR METAHEXAMIDE AND CAPILLARY VENOUS DIFFERENCES FOR GLUCOSE IN ELDERLY DIABETIC MEN USING INSULIN OR METAHEXAMIDE*

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A significant hypoglycemic action has not been demonstrated for the sulfonylureas in depancreatized man[1-7] or other mammals[7-9] nor has it been observed in completely alloxanized animals.[1, 9-13] This and other evidence[4, 14-20] support the hypothesis that an important action of the sulfonylureas is an increase in the production, release, or both, of insulin by the pancreas. On the other hand, in the fowl the peripheral utilization of glucose has been demonstrated in the absence of both the pancreas and the liver.[21, 22] Employing arteriovenous (A-V) glucose differences and the ratio of A-V glucose differences to arterial blood glucose (A-V/A), Madison and Unger[23] have demonstrated comparable uptakes of glucose by peripheral tissues in normal fasting dogs after the administration of insulin and tolbutamide. Their data "support, but do not prove, the thesis that one of the major physiologic effects of tolbutamide is the stimulation of endogeneous insulin secretion"

The experiments described in this paper were planned to compare the effects of insulin and metahexamide upon the peripheral utilization of glucose by measuring capillary venous (C-V) differences and by the claculation of the ratio of C-V glucose differences to capillary blood glucose (C-V/C).

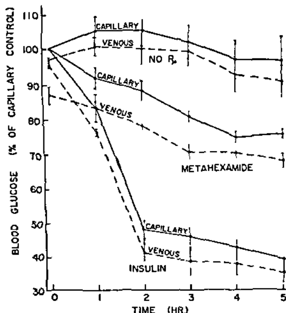
Methods and Procedure

Five elderly (range 63 to 75 years, with an average of 66 years) ketoacidosis-resistant diabetic men, requiring from 10 to 40 U. of insulin daily (average, 26 U.) to maintain reasonable control were stabilized as to balanced diet and weight for at least 2 months prior to the study. A staisfactory transfer from insulin to metahexamide had been made 38, 29, 12, 93, and 55 days (average, 50 days) prior to the first test in cases 4, 7, 8, 10, and 11, respectively † Until the day prior to the metahexamide tests

[illegible] itely following the [illegible], each subject was [illegible] re taken thereafter at hourly intervals for 5 hours while the patient fasted (TABLE 1) No further doses of metahexamide were used until completion of similar determinations in (1) patients given no medication (carried out 4 or 5 days after the metahexamide study) and (2) in patients administered insulin in the dosage employed by the individual before any treatment with metahexamide (carried out 3 days

† [illegible]

used. During this time, the fall of blood sugar was much more marked with insulin than with metahexamide (TABLE 1 and FIGURE 1) In all instances, however, patients receiving either metahexamide or insulin showed significantly greater decreases in blood sugar than were observed in patients who had



diabetic men and 8 nondiabetic subjects (TABLE 2). Sixteen sets of values were obtained in connection with a dose of 200 mg , 5 with a dose of 500 mg , and

direct correlation between the size of the dose and the time required for the blood level to reach half value The half value was reached at the 800-mg dose level somewhere between 24 and 48 hours; at the 500-mg. dose level between 20 and 24 hours, and at the 200-mg dose, between 16 and 20 hours.

following the determinations made during "fasting without medication"). Until all 3 test days were completed, regular insulin was used as necessary to maintain reasonable control of the diabetic state, but in no instance was this administered later than 15 hours before starting a series of blood determinations, nor was such supplemental insulin required frequently.

To minimize glycolysis, protein-free filtrates were prepared immediately after the withdrawal of the blood specimen. The determinations of glucose were made by the Somogyi-Nelson method.[25] From aliquots of the specimens removed for the estimation of glucose, the blood levels for metahexamide were determined by the method of Bratton and Marshall,[26] with this slight modification: before the addition of the coupling reagent, 1 cc. of concentrated hydrochloric acid was added to make the resulting dye soluble.

Results

C-V glucose differences and C-V/C ratios. Taking into consideration the age of our subjects, it may be seen from TABLE 1 that 2 of the 5 exhibited satisfactory blood glucose values at the beginning of the metahexamide tests, 2 showed only moderately elevated values, and 1 exhibited a high value. However, none showed more than 1+ glucose in the urine, and in no instance did more than 2 of the 4 daily fractional urines contain glucose. Metahexamide in maintenance dose had been continued until the day before the metahexamide test. On the morning of the metahexamide tests, the premedication C-V differences were greatest for those subjects who showed normal or nearly normal blood sugars, probably indicating a continuing activity of the metahexamide given the day before. Four or five days later, the fasting blood sugars were not greatly different from those observed at the end of the metahexamide regime. An attempt had been made to preserve about the same degree of control through the use of regular insulin when indicated by the appearance of more than 1+ glycosuria. During the interval between the determinations "without medication" and the test made following a single dose of insulin, (comparable to that used in the given individual before transference to metahexamide therapy), 2 of the patients showed a significantly higher fasting glucose level, while a slight elevation was present in 2 of the other 3 subjects. This occurred in spite of our efforts to maintain, by the use of regular insulin, approximately the same control as before.

As in A-V differences observed by other workers,[23, 27, 28] there was considerable variation in the C-V differences from patient to patient in the present studies. However, when averages were made and the standard deviations considered, it was noted that both insulin and metahexamide administration were associated with an increase in the C-V differences. We believe the higher premedication zero-hour C-V differences for metahexamide represented a continuing influence of maintenance doses used up to and including the morning before the test. In regard to the high zero-hour value for the insulin test, this average is comparable to that in the "without medication" test, if we exclude the 1 patient who had 2 doses of insulin on the preceding day. From the first through the third hours, it appears that the C-V differences were increased about equally by metahexamide and insulin in the dosages

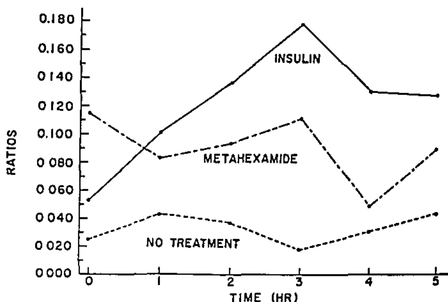
Discussion

One acceptable parameter of peripheral glucose utilization has been the A-V glucose difference, although attention has been called to considerable variability in these values from subject to subject under the influence of insulin.[23,27,28] However, Madison and Unger have demonstrated that, in normal dogs, the responses of the individual animal to insulin and to tolbutamide are comparable In other words, the animal that develops a striking hypoglycemia to insulin, a wide A-V difference, or both, will show a similar response to tolbutamide This relationship did not exist for insulin and metahexamide under the conditions of our experiments *in older, mildly diabetic men* Nevertheless, there seems to be little doubt that the peripheral utilization of glucose was furthered by metahexamide in these subjects It is unlikely that these *C-V differences alone accounted for the degree of hypoglycemia produced* The data are in accord with those of other workers,[23,29,30] namely, that in the

Inasmuch as capillary blood is actually a mixture of arterial and venous blood, the changes observed become even more striking, as arterial-venous differences would probably have been greater Moreover, the C-V differences obtained represented an uptake of glucose chiefly by the skin and subcutaneous tissues, whereas A-V differences reflect the activity of all skeletal tissues, including muscle

That some workers[6,31-33] have failed to obtain data supporting evidence for the peripheral utilization of glucose may be due to differences in methods and experimental design Many of these features have been dealt with at length by others[23] Aside from A-V differences, other evidence for the peripheral activity of the sulfonylureas has been adduced Ashmore and his associates[34] and W L Miller and his colleagues[10,35] have shown that under certain conditions tolbutamide will increase the formation of glycogen in muscle, although to not as marked a degree as insulin Miller and his co-workers believe this action has been overlooked by a majority of observers because most of the experiments designed to prove or disprove this action have employed improper doses or insufficient periods of observation

Because degradation of metahexamide occurs chiefly by hydrolysis and not by acetylation,[36] its removal from the blood is comparatively slow This too may be the reason why the rate of removal of metahexamide is more dependent *upon dosage than is the rate of removal of tolbutamide.* Most observers give the half-time value as 20 to 22 5 hours, this figure being used irrespective of doses varying from 0 5[36] to 2 0 gm[37] Our studies indicate dosage as an important *factor in the half-value time of metahexamide In view of the fact that* disappearance of a single dose is rarely complete in any subject after less than 4 days, a cumulative action must be reckoned with in relation to continuing doses, this should be considered in any final adjustment of maintenance of the diabetic patient taking metahexamide.



tained

TABLE 2
METAHEXAMIDE BLOOD LEVELS IN 21 SUBJECTS AFTER ORAL ADMINISTRATION
mg/100 ml

Hours	Dose (mg)		
	800	500	200
1	1.5 (0.3–2.5)*	2.2 (0.78–2.64)*	—
2	3.0 (2.9–3.2)	2.7 (1.10–3.47)	1.7 (0.7–3.0)*
3	3.9 (3.3–4.3)	3.2 (1.82–3.74)	—
4	—	3.0 (1.28–3.61)	1.6 (0.8–2.9)
5	—	2.9 (0.99–3.53)	—
6	—	—	1.5 (0.8–2.9)
8	3.7 (3.3–4.0)	—	1.3 (0.7–2.7)
12	—	—	0.9 (0.5–1.1)
16	—	—	1.1 (0.6–1.2)
20	—	1.8 (0.82–2.72)	0.8 (0.6–1.0)
24	2.7 (2.4–2.9)	1.3 (0.42–1.89)	0.8 (0.3–2.1)
48	1.7 (1.6–1.9)	—	—
72	1.1 (1.0–1.3)	—	—
96	0.8 (0.7–0.9)	—	—
120	0.2	—	—
192		0.2 (0.0–0.5)	

* Figures in parentheses give the range of values

Discussion

One acceptable parameter of peripheral glucose utilization has been the A-V glucose difference, although attention has been called to considerable variability in these values from subject to subject under the influence of insulin[23, 27, 28] However, Madison and Unger have demonstrated that, in normal dogs, the responses of the individual animal to insulin and to tolbutamide are comparable. In other words, the animal that develops a striking hypoglycemia to insulin, a wide A-V difference, or both, will show a similar response to tolbutamide. This relationship did not exist for insulin and metahexamide under the conditions of our experiments in older, mildly diabetic men Nevertheless, there seems to be little doubt that the peripheral utilization of glucose was furthered by metahexamide in these subjects It is unlikely that these C-V differences alone accounted for the degree of hypoglycemia produced The data are in accord with those of other workers,[23, 29, 30] namely, that in the presence of some pancreatic function or exogeneous insulin, a portion of the action of mtahexamide is concerned with the peripheral utilization of glucose However, this does not account for the full effect

Inasmuch as capillary blood is actually a mixture of arterial and venous blood, the changes observed become even more striking, as arterial-venous differences would probably have been greater Moreover, the C-V differences obtained represented an uptake of glucose chiefly by the skin and subcutaneous tissues, whereas A-V differences reflect the activity of all skeletal tissues, including muscle.

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Summary and Conclusion

C-V differences for glucose have been determined in 5 elderly diabetic men in the fasting state and after being administered insulin (26 U.) and metahexamide (500 mg). There was no significant variation between the averaged differences obtained for metahexamide and for insulin, but both were significantly greater than the values obtained during fasting. In the doses used, the hypoglycemic action of insulin was greater than that of metahexamide.

Blood level half-value times determined for metahexamide showed that these varied directly with the size of the dose, but not in linear fashion.

Metahexamide is a potent hypoglycemic agent, a portion of the action of which is exerted, either directly or indirectly, upon the peripheral tissues.

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SUMMARY OF THE MONOGRAPH

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It is questionable whether the material presented in this monograph should be summarized at all, since this would of necessity provide a static ending to . However,

as definitely

the prevalence of diabetes mellitus in the United States today appears to be approximately 19 per 1000 population, with only about one half of this number being recognized This very fact underlines the continued need for developing ever more stringent criteria for what constitutes "normal" regulation of carbohydrate metabolism and improved methods of detecting decreased carbohydrate tolerance as a means of recognizing the disease earlier This aspect of the problem is discussed by Fajans and Conn. It will be apparent that such research

with

years

and thus affords a spontaneous test for any diabetic tendency as manifested by certain abnormalities in both the fetus and the newborn. Preliminary

birth, and that the same percentage gives a family history of diabetes. Since either insulin or tolbutamide treatment has been shown to return fetal mortality to normal, such investigations are of importance not only from the ecological point of view, but also in terms of prevention.

In addition to our concerted efforts to safeguard the survival of diabetics from their intrauterine existence onward, there is also an ever more thoughtful approach toward evaluating diabetics seeking employment. Generally acceptable definitions of the degree of control and permissible commitments have been devised and, according to Brandaleone, the diabetic is now generally accepted by industry as a reliable worker The acceptance of such patients for life insurance is still rather limited, but Entmacher finds that more policies are being offered, although at least twice the normal premium is usually required. The principal reason for this lies in the accelerated vascular and neurological degenerative diseases that accompany diabetes, certain aspects of which have been discussed by Bloch and Ellenberg

As diabetes is characterized most simply as an absolute or relative lack of insulin, islet-cell function is of crucial importance Haist's broad discussion of the factors enhancing insulin production and secretion by the beta cells of the islets of Langerhans summarizes the field For the growth of islet cells, anterior pituitary growth hormone appears to be essential. Insulin secretion

Prematurity-onset diabetes is accompanied by an absolute lack of insulin, whereas postmaturity-onset diabetes is usually characterized by normal insulin levels In the latter type of the disease one is dealing with a subnormal islet cell response to a natural stimulus, namely, an elevated blood sugar level. There is failure of release of insulin, rather than of its synthesis, and it is in this group that the sulfonylureas appear to afford a beneficial therapeutic approach Although the diabetic state is generally considered to be irreversible, one should not give up hope that, with a better understanding of the factors involved in islet-cell growth and insulin synthesis, at least partial re-establishment of the islet-cell function eventually may be achieved even in diabetics with insufficient islet-cell tissue Development of new beta cells from ductal tissue in the rabbit given hyperglycemic agents is beautifully illustrated in the presentation of Volk and Lazarus The electron-microscopic pictures of the beta cells shown by Lacy and Hartroft reveal the great variety of particulate organization in different species and should serve as the basis for a better understanding of the influence of various hormones on islet-cell function *Batts' ingenious use of changes in the Golgi apparatus as a means* of demonstrating the secretory activity of the beta cells affords an elegant technique for further physiological study

The fate of insulin, once released from the islets, has been investigated by the use of I^{131}-labeled insulin The presentation of Unger and his associates

tially only a hepatic effect without affecting peripheral metabolism The basic findings are well confirmed in the studies of Mortimore and Tietze in the rat, using a more direct approach of liver perfusion *in situ* and demonstrating considerable inactivation of the insulin by liver tissue The one serious limitation of both of these studies lies in the use of insulin from other species and the changes in the insulin molecule brought about by I^{131}, however small the quan-

more studies on desensitization procedures by modern methodology. The theoretical advantage of the use of sulfonylureas in this respect are obvious.

The exact mechanism whereby insulin acts on the intermediary metabolism of the cell still remains a problem requiring much active work. For many years the rat diaphragm has served as a useful model of peripheral muscle tissue for the investigation of insulin action The paper of Larner and his co-workers demonstrates the effective use of this technique in demonstrating

glycogen synthesis independent of inorganic phosphate. Kipnis has introduced a special technique for removing the diaphragm that obviates cutting the tissue and thereby damaging it. Utilizing the classical *cut* preparation to measure phosphorylating capacity of muscle and the *intact* preparation, he shows that insulin affects primarily the rate of glucose transfer through the cell membrane, whereas adrenocortical hormones, pituitary growth hormone, and epinephrine decrease glucose tolerance by diminishing the rate of intracellular phosphorylation of glucose.

The studies by Park and Morgan and his associates on the effect of insulin and other agents on glucose utilization by the isolated rabbit heart, supplement the observations by Kipnis on the diaphragm of the rat. Including studies in both the aerobic and anaerobic state, these investigators show that the regulatory mechanisms for transport appear to be insulin and the level of aerobic metabolism, whereas the factors that regulate phosphorylation are the pituitary growth hormone and the adrenocortical secretions. The more anaerobic the environment, the faster both the penetration and the intracellular phosphorylation.

Developments in the glucose-transport theory of insulin action are succinctly

amine synthesis by the liver. This finding is somewhat surprising, since hepatic glucoseamine is a constituent of mucopolysaccharide and, as pointed out in discussion, the latter has been shown to be synthesized at a much diminished rate in the skin of the diabetic rat, explaining poor wound healing and, possibly, the small-vessel disease.

Cahill and his collaborators summarize much of their work on the effects of insulin on adipose tissue, now known to be very active metabolically, controlling glucose uptake, oxidation, and transformation into glycogen and triglyceride. The demonstrated blocking of the release of free fatty acids by insulin illustrates an important antiketogenic mechanism, and the effect on glycerol metabolism opens new vistas in intermediary metabolism.

The effect of insulin and tolbutamide on hepatic glucose release is covered in five papers: those of Steele, of de Bodo *et al.*, of Unger *et al.*, of Mahler *et al.*, and of Frawley *et al.* Utilizing various techniques, laboratory animals in different states of consciousness, and varying interpretations, two different concepts arise from this work: first, that small amounts of insulin reduce the secretion of glucose from the liver (Unger *et al.*, Reichard *et al.*, and Frawley *et al.*), and, second, that insulin has no effect on the hepatic output of glucose (Steele, Mahler *et al.*, and de Bodo *et al.*). The simultaneous use of arteriovenous differences in glucose across the hepatic bed and glucose-C^{14} dilution studies might some day resolve the differing points of view.

far the safest and least toxic of the oral hypoglycemic agents Chlorpropamide appears to be 4 to 6 times more potent, and this enhancement is almost entirely due to its longer active half life in the tissue fluids This characteristic, of course, has both advantages (convenience) and dangers (hypoglycemia) attached to it The effectiveness of this drug in cases refractory to tolbutamide is of doubtful significance, since in some reported cases the potency differential may have been responsible rather than the different nature of the drug The acceptance of the beta-cytotropic theory of action of the sulfonylureas limits *their use to postmaturity-onset cases, this is not the case with the biguanides* However, the latter drugs appear to have a somewhat limited usefulness because of their tendency to give rise to gastrointestinal upsets as the dosage is increased Used jointly with insulin in "brittle" diabetics, there is a definite indication in selected cases

Metahexamide received considerable discussion by a variety of authors, but in both the papers and the discussions an undertone of apprehension was noticeable lest the early indications of toxicity, including hepatic necrosis, might eventually limit the usefulness of this new agent Subsequent to the conference upon which this monograph is based the manufacturers of this compound withdrew it from experimental trial because of the rapidly mounting number of serious and, *in some instances, fatal liver involvement*

There are thus, at the time of this summary, four therapeutic agents available in the therapy of the various types of diabetes Insulin is still the mainstay for diabetic control in the prematurity-onset type and is used to advantage in many middle-aged patients, especially those who are underweight Tolbutamide is the safest and most widely used oral hypoglycemic agent for control of patients with postmaturity-onset diabetes, using less than 40 units of

esses and of the changes brought about by the diabetic state By bringing together basic and practical considerations, a publication such as this will, we believe, benefit a large number of laboratory workers and clinicians alike

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